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ANNALS
OF
TROPICAL MEDICINE AND
PARASITOLOGY

ISSUED BY THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

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E. A. Minchin

ON THE 'VOMITING SICKNESS' OF JAMAICA

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(Received for publication 25 October, 1915)

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I. INTRODUCTION

The so-called Vomiting Sickness of Jamaica has been a veritable scourge in this island for many years past. It rages during the cooler months of the year, starting towards the end of November or beginning of December and continuing its ravages to the end of March or well into April. The mortality rate is very high, and in some years it takes a toll of deaths which runs into hundreds. During the last season, December, 1914 to April, 1915, the prevalence has been exceptionally widespread and severe, and I had the good fortune to be able to study on the spot a very fatal outbreak on the north side of the island. As a result of that investigation I have undertaken certain experimental work—described in this paper—to clear up the mysterious question of the causation of this peculiar and interesting disease.

II. HISTORICAL

The earliest recorded statements concerning this peculiar affection which I have been able to find are those made in January, 1900, by Dr. R. G. S. Bell, District Medical Officer of May Pen, and by Dr. H. G. Tillman, at Vere, in February of the same year.

Other records, between 1900 and 1905, were given by Drs. Ker, J. A. L. Calder, Edwards, Turton, Cooke, A. W. Thomson, and Earle.

During the following year (1905-6) the disease was much less extensive and prevailed for a shorter period, viz., December to February. It occurred most at Newport, a district which had not been mentioned in the previous outbreak.

The year 1906 is an important one in the history of the affection, for a letter was addressed to the authorities of other West Indian Islands, describing the disease and enquiring whether anything similar was prevalent in those localities. Replies were mainly in the negative; two only are quoted as of interest.

(i) Dr. Carlos Finlay (1906) mentions an outbreak which he calls cerebro-spinal meningitis, with five fatal cases, occurring in the troops of an American regiment near Mariano in Cuba. This is certainly not the same as the vomiting sickness of Jamaica, for it attacked adults only and did not recur after the single outbreak in 1899. Also, as will be seen later, the symptoms and course of cerebro-spinal meningitis differ from those of typical vomiting sickness.

(ii) The Consul-General in Haiti (1906) wrote: 'There is a disease somewhat similar in this island. It has not, however, proved to be particularly dangerous. It generally happens during the months of February, March, and April, when there is a difference in temperature by day and by night. It can best be described as severe bowel troubles, together with high fever and vomiting. Europeans suffer principally from it, and natives to a much less extent. Children are apt to suffer from it, but there does not appear to be any high mortality from it. The attack (acute stage) lasts on an average from three to five days, when improvement sets in with rapid convalescence.'

It will be seen from a perusal of the following pages that this

condition is quite different from the vomiting sickness of Jamaica. Briefly, the differences may be tabulated thus:—

<i>Haitian Disease</i>	<i>Vomiting Sickness</i>
1. Bowel troubles.	1. No bowel troubles, as a rule.
2. High fever.	2. Hardly any fever; temperature may rise to 99·4°, seldom reaches 100° F.
3. Europeans principally attacked, natives less.	3. Europeans practically never, natives about 99 per cent.
4. Mortality not high.	4. Mortality about 80 per cent.
5. Attack (acute stage) lasts three to five days.	5. Death usually in 15 hours, may occur within an hour. If attack lasts 24 hours, there is nearly always recovery.

This is mentioned in some detail because one of the chief points to be explained (see later) is the confinement of the disease, so far as is known, to Jamaica.

Replies were also received from Cuba, Nassau, Trinidad, Grenada, Barbadoes, Santi Domingo, British Guiana, Antigua, St. Vincent, St. Lucia and Porto Rico; so that we may infer that the inhabitants of the other islands do not suffer from any disease corresponding to the vomiting sickness.

In the 1907 report it is noted that there had been that year 'more than ordinarily severe outbreaks of this disease.'

The reports for 1908 and 1909 give very meagre statements as regards this disease.

In the 1909 report there are only casual remarks upon the disease.

In 1910 the disease was fairly widespread and severe.

The following year, 1910-1911, must be dealt with in greater detail, since, in consequence of communications from His Excellency, the Governor, Sir Sydney Olivier, the Research Committee of the Colonial Office selected Captain (now Major) T. J. Potter, R.A.M.C., to carry out investigations into this disease. He arrived on Christmas Day, 1910, and remained in the island till August, 1911. In consequence of his being present and knowing that a report would follow, the various medical officers made but very meagre remarks on the subject for that year.

Potter's report was published in 1912, and he was of the opinion that the majority of deaths ascribed to the so-called vomiting sickness were due to yellow fever.

Eighteen months ago (February, 1914), when writing on this same question, I stated (Scott, 1914): 'Of nearly 200 cases reported to me in detail, only two had any vomiting so described, and amongst those seen by me personally I have never met with a case in which the vomit was black. . . . The vomitus in all that I have seen has been in the main mucoid, watery, or frothy, while very occasionally, if there has been much straining or retching, it may be pinkish from admixed blood.'

Dr. Seidelin (1913) was struck by the rarity of black vomit.

We now come to what may be called the 'meningitis era' of the history of the investigation, in reality a recrudescence of the older theory that vomiting sickness and cerebro-spinal meningitis were one and the same. This period was intermediate between the investigation of Captain Potter and that of Dr. Seidelin.

Though cases of meningitis occur amongst those reported as vomiting sickness, the two diseases are not, I believe, identical. After the former have been excluded, a large proportion still remains unaccounted for, so far as actual knowledge of the cause goes at present.

Meanwhile, in January, 1913, Dr. Harald Seidelin arrived from England, having been sent out by the Liverpool School of Tropical Medicine to investigate the disease. His report (Seidelin, 1913) with notes and analyses of 62 cases appeared in the *Annals of Tropical Medicine and Parasitology*, Vol. VII, pp. 377-478.

The great value of this report consists in the fact of its containing such excellent accounts of the morbid anatomy of the disease. The post-mortem appearances, both macroscopical and microscopical, are carefully described in detail.

In the year subsequent to that of Seidelin's investigations very few cases occurred.

III. PERSONAL OBSERVATIONS ON THE MONTEGO BAY OUTBREAK, FEBRUARY, 1915

Such was the state of the question at the beginning of 1915. It will be seen that various diagnoses had been given from time to time—gastritis, gastro-enteritis, worms, malaria, pneumonia, cerebro-spinal meningitis, yellow fever, and so on.

In the sense that possibly all these had at one time or another

been included under the general heading of vomiting sickness we might say, in the words of Kipling, 'Every single one of them is right.' As has been already stated, during the cooler months, any case of a child who dies after having vomited, unless there is some very definite cause, is diagnosed as vomiting sickness; while others, dying from some obscure condition, if vomiting has not occurred, are diagnosed as 'vomiting sickness without vomiting.'

In short, the name of 'vomiting sickness' has been used as successfully as 'charity' in covering a multitude of sins. Apart from those mentioned above, among the number reported to me as cases of vomiting sickness I may mention such as proved to be marasmus, intussusception, status epilepticus, cerebral haemorrhage, pregnancy. Truly a chaotic state of things this, when the epidemic of 1915 began.

During this period the disease was very rife, more widespread and more prolonged. An exceptional opportunity of studying the condition on the spot arose in February, when Dr. Thomson, the District Medical Officer of Montego Bay, reported a severe outbreak with eighteen deaths in two days. I went immediately and stayed in the district, and was able to see several cases, some of them almost from start to finish. In company with the District Medical Officer, who had kept records of most of the cases, I was enabled to interview survivors or the relatives of those who had died. I visited the huts where the cases had occurred, and performed autopsies on all who died during my stay in the neighbourhood. I obtained detailed notes of thirty-two cases of true vomiting sickness, nearly every one conforming to the standard type of the disease. Three others were reported as such, but one turned out to be acute haemorrhagic pancreatitis, one was a case of ordinary infantile convulsions without the usual accompanying symptoms of vomiting sickness, the third was a patient suffering from ordinary malaria of fairly long standing, not an acute case at all. *Plasmodium falciparum* was seen in her blood, and she made a complete recovery on the usual antimalarial lines of treatment.

Since this outbreak and the investigations resulting from it appear to furnish the key to the problem, and as nearly every case brings a certain weight of corroborative evidence, the cases must be given in some detail; detail, that is, as regards history only, as I do not

wish to burden this section with pathological findings, which have their place subsequently.

CASE 1. S.M., female, 12 years of age; living at 'Retirement.' On February 16th went to school perfectly well. About 4 p.m. complained of pain in the stomach, and vomited three times. Felt better and tried to get home, but on the way felt ill and again vomited, and finally reached home between 7.30 and 8 p.m. Shortly afterwards she passed into a state of coma and died about midnight. Nature of previous meal not known; many ackee trees with ripe fruit in the yard.

The following eleven cases occurred at 'Salt Spring':—

CASE 2. D.C., female, 6 years. Well on going to bed on February 16th. At 5 a.m., 17th, vomited twice, but did not complain of any pain. Felt sick all day 17th; at 6 a.m., 18th, after a fairly good night, vomiting started again; almost immediately afterwards she had a convulsion and passed into a state of coma, in which she died at 2 p.m.

Her food consisted of yam, banana, and 'probably ackee,' according to the mother. There were many in the yard, but the mother would not say for certain whether the child had eaten them.

CASE 3. N.R.D., male, 10 years. At 5 p.m., February 17th, complained of pain in the stomach, and began to vomit; continued to do so until 1 a.m., 18th, when convulsions set in, followed by coma which lasted till death, at noon.

The food taken at 3 p.m., 17th, had consisted of yam, salt-fish, ackee, and bananas, all boiled together. The ackee was mostly removed and eaten by the older members of the family (Nos. 5, 6, and 7), leaving the 'soup' or 'pot-water' for this child, and the one whose case follows.

CASE 4. M.C., female, 13 years, cousin to the last, and living in the same hut. Vomiting began about the same time, 5 p.m., and ceased at midnight; returned about noon, 18th, and lasted till 3 p.m. There were no convulsions, but coma with restlessness; death took place at 3 a.m., 19th.

Food: part of the same meal as that of the last patient, viz., the 'soup' of the yam, bananas, ackee, etc., which had been boiled together.

CASE 5. R.C., female, 26 years, mother of N.R.D. (No. 3), and aunt of M.C. (No. 4). Vomiting began at 5 p.m., 17th, and continued all night. It ceased in the early hours of the 18th. Feeling better she took some more of the same articles during the day as had been boiled for the family on the 17th. The vomiting started again the same evening and continued during the following day. This patient recovered.

CASE 6. J.J., female, 65 years; lived with the last three, and was the mother of R.C., and grandmother of N.R.D. Vomiting began about 8 p.m., 18th, and continued till 2 a.m., 19th. During the 19th she vomited at intervals, but eventually recovered.

This woman partook of the same meal as the others (yam, bananas, salt-fish, and ackee).

CASE 7. B.J., female, 21 years; another daughter of the last. Vomiting began during the evening of the 18th and continued at intervals for 24 hours. She also recovered.

Her food was the same as that of the four previous cases.

An important point to note in this series of cases is that the same

food was used by all; the older members ate the solid and recovered, the younger were given the 'soup' or 'pot-water' and died. Also R. C. (No. 5) showed a return of the symptoms on again partaking of a similar diet.

CASE 8. F.W., female, 8 years. On February 17th she had a meal of 'yam, banana, pumpkin, and possibly ackee' (parents' statement) all boiled together. There were many ackees in the yard. Vomiting began the same evening, and shortly afterwards convulsions and coma supervened, and she remained comatose till death, at noon on the 18th.

CASE 9. H.W., male, 1 year and 9 months; brother to the last-named. Was apparently quite well on going to bed on the 17th. About dawn, 18th, complained of pain in the stomach; soon after was attacked by convulsions, became comatose, and died at 8 a.m. His food was the 'soup' or 'pot-water,' i.e., the gravy or liquid from boiling the above articles of food.

CASE 10. P.C., female, 25 years; mother of the last two. Meal on the 17th consisted of yam, bananas, pumpkin, and ackee. Early on the 18th, 'about dawn,' suffered from griping pains, vomiting and diarrhoea. These symptoms continued all day, and she felt so ill that she could not even attend to the two children who were dying. The symptoms subsided the next day, and she rapidly recovered.

The history as regards the nature of the food in this case was quite definite, and tends to prove the presence of ackee in the meal of the two children. It is incredible that a poor woman, such as this was, would cook two similar breakfasts, one for the children and another for herself, one of yams, bananas, and pumpkin, and the other of yams, bananas, pumpkin, and ackees. Probably, nay, almost certainly, all four ingredients were cooked together, as is customary, the ackees being picked out and eaten by the mother, the more solid residue by the older child, the 'pot-water' being given to the younger.

Note the graded acuteness of the illness: the mother, who had the solid ackees, was seriously ill for thirty-six hours or so, and recovered; the older child partook of the other ingredients, with, of course, some of the absorbed 'pot-water,' was ill for about sixteen hours, and then died; the younger, who had the 'pot-water' only, was acutely ill, and died in about two hours.

CASE 11. R.M., female, 4 years. At 10 a.m., February 18th, was seized with convulsions (there may have been vomiting previously, but this was uncertain), rapidly became comatose, and died at 2 p.m.

This case was reported as one of vomiting sickness and is, therefore, included here. The post-mortem findings were those of vomiting sickness, but otherwise it might be merely a case of infantile convulsions of gastric or intestinal origin associated with worms, for many *Ascarides* were present.

Food: uncertain; said to be 'pap,' but the child was running about, and there were many ackees in the yard, and she could easily have picked up an unsound one and have eaten it.

CASE 12. I.F., female, 4 years. Apparently well at 3 p.m., February 20th, when she lay down and went to sleep. An hour later her mother tried to awaken her, but could not. On stimulation the child seemed to revive a little, but

shortly afterwards there was twitching and a slight convulsion, with coma, which deepened till death, at 1 p.m. on February 21st.

The food consisted of yam, bananas, and 'possibly ackee.' The mother was 'not quite certain' whether the patient ate any. There were large numbers in the yard.

The following case occurred at Green Pond, Portobello, near Salt Spring:—

CASE 13. A.R., female, 2 years 10 months. At 11 a.m., February 24th, a meal, consisting of pumpkin, yam, peas, and ackees, all boiled together, was prepared for the family. This child was given the 'pot-water.' Two hours later she complained of pain in the stomach, and at 1 p.m., after vomiting twice, she went to lie down. Vomiting increased in frequency till 2 p.m., when muscular twitchings and convulsive attacks supervened, the child lost consciousness, and died comatose at 5 p.m.

The next six cases occurred at Montego Bay itself.

CASE 14. G.G., male, 8 years. At noon on February 17th a meal was prepared, which consisted of yam, bananas, and ackees, all boiled together. The parents stated that they themselves ate the ackees, while the patient and his sister (No. 15) were given the 'pot-water,' and some of the other ingredients. He started to vomit at 3 p.m., and continued to do so till 9 p.m., when convulsions came on, succeeded by coma, which terminated fatally at 2 a.m., 18th.

CASE 15. D.G., female, 11 years; sister to the last. Vomiting started during the night of the 17th and continued till the morning when convulsions and coma came on, and death occurred at 10 a.m., 18th.

The history as regards food is the same as the last.

These two patients had accompanied their parents to their provision ground, a mile away, where the meal was cooked.

CASE 16. W.G., male, 9 years; and

CASE 17. A.G., female, 4 years; brother and sister respectively of the last two. These were away from the rest of the family during the day. The history of both was the same. Vomiting, not very severe, and not accompanied by convulsions or loss of consciousness, began in the morning of the 18th. Both recovered in 24 hours.

The parents were not very sure about the food in these two cases, while they were away. There were many ackee trees in the yard, where they stayed all day, and also the parents thought that they had had some of the remains of the food left over from the meal described above—yam, bananas, and ackees.

These four cases are interesting. They all belong to the same family. Two undoubtedly were given ackee or ackee-water (pot-water); the other two had probably eaten some. Those who were known to have had the 'soup' or 'pot-water' died, while the others suffered from vomiting, but recovered.

CASE 18. B.R., female, 14 years 11 months. Started to vomit at 4 p.m. 17th, and continued to do so during the night. In the course of the following morning she became unconscious, and remained comatose till death on the 19th.

As regards the question of the food in this case. All the statement obtainable

from the mother was 'ordinary food,' and when pressed for details would only say 'yam and beef.' This was exceedingly unlikely; the place was poor, the child emaciated, beef would be a great luxury, and in such a state of poverty ackees would be certainly used as they were plentiful in the yard. Moreover, 'methought the lady did protest too much' against the idea of the girl having eaten any.

CASE 19. P.R., male, 6 years. At 5 p.m., February 24th, was given a dinner of salt fish and ackee. At 7 p.m. he suddenly began to vomit, but did not complain of any pain. He vomited up ackee. During the night he improved, but at 5 a.m. he had a fit and became unconscious. He continued in this state, but with deepening coma, till death at 3 p.m.

The next twelve cases occurred at 'Granville,' another sub-district of Montego Bay.

CASE 20. W.B.H., male, 5 years. Early on February 18th he began to vomit, but made no complaint of pain. The vomiting continued for an hour, when convulsions supervened, and the child passed into a state of coma, and died about 4 hours after the onset.

Food: see after next case.

CASE 21. G.S.H., male, 2 years 10 months; brother to the last. Similar history, but the duration of the illness was 5 hours.

Both children spent the previous day with their grandmother. This woman stated that she gave them yam and bananas, but there were many ackee trees with ripe fruit in the yard, and many ackees were lying on the ground. She denied having given any to the children, but owned that they might easily have picked them up and eaten them.

I am inclined to think that this is more likely, because the delay in onset would then be explained by the fact that only a small part of the fruit might be toxic (some soft part, for example), and being solid took longer to be absorbed. There was, it will be noted, very little local action; there was no complaint of abdominal pain, but almost entirely nervous symptoms.

CASE 22. A.M.H., female, 8 years; sister to the last two. Began to vomit at 10 a.m., 18th, and continued to vomit for two to three hours. About 1 p.m. convulsions set in, followed almost at once by coma, which lasted till death, between 6 and 7 p.m.

This child had also been for a short time to the grandmother's hut. Her mother stated that she had given her a meal of yam, bananas, salt-fish, and 'possibly a little ackee.' (See next case.)

CASE 23. A.H., female, 42 years; mother of the last three. Had a severe attack of vomiting during February 18th, starting at 6 a.m.; she then felt faint and giddy, but had no convulsions, and did not lose consciousness. She made a good recovery.

This woman definitely stated that her food on the day in question, 17th, consisted of yam, bananas, and ackees. Seeing that the daughter, A.M.H., was with her practically the whole day, and took her meal with her, the former almost certainly had ackees too; for, as mentioned in the series 8, 9, and 10, it is most unlikely, to say the least, that the mother would cook two dinners of the same ingredients—yam and bananas—and place ackees in one and not in the other. More probably, as appears to be customary, the mother ate the ackees herself, and the child had some of the yam and bananas with the 'soup' or 'pot-water.'

The latter's attack terminated fatally, while the former suffered from gastric symptoms mainly, with some vertigo, and recovered.

CASE 24. J.McB.B., male, 6 years 9 months. On February 17th, was given a meal of yam, bananas, and ackees, boiled up together. Early on the 18th he appeared 'droopy and dull.' During the day the 'soup' of a similar meal was given to him, but he only took a little of it, and the rest of the liquid was thrown away. At 5.30 p.m. vomiting came on, convulsions followed almost at once, with a condition of deepening coma which lasted till death, at 4 p.m., on the 19th.

CASE 25. L.R., female, 26 years; mother of the last. Began to vomit at 9 a.m., 19th, and vomited several times between then and 3 p.m. Vomiting ceased then, and she made a good recovery. She had no convulsions and never lost consciousness.

Her food was the same as that just mentioned—yam, bananas, and ackees—of which she took the solid, the liquid being thrown away after being refused by the child. It will be seen that the mother ate the solid ingredients and so much of the liquid as had soaked into them; she vomited and recovered; whereas the child had some of the 'soup' and died.

CASE 26. C.S., female, 25 years. Vomited on and off during February 18th, and late in the afternoon was seized with convulsions, and lost consciousness. She remained comatose till death on the following day.

Nothing could be discovered regarding this patient's food, but it may be noted that she lived in the same yard as the case, No. 24, already related, where ackees were numerous.

CASE 27. E.B., female, 13 months. Began to vomit in the evening of February 19th; was almost immediately attacked by convulsions, passed into coma, and died at 4 a.m., 20th.

Food: 'not known, probably pap,' (mother's statement). But the child was accustomed to crawl about unattended and unwatched, and there were ackees lying about in the yard.

CASE 28. C.W., female, 8 years. Began to vomit at 2 p.m., 20th; vomited matter appeared to consist of yam and banana. About 6 hours later coma supervened, without any convulsion, and she died without regaining consciousness.

CASE 29. S.W., female, 6 years; sister to the last. Vomiting started about 3 p.m. the same day, but was not severe. There was no convulsions nor loss of consciousness, and the child recovered within 24 hours.

Nothing could be ascertained with certainty about the nature of the food in either of these cases, as they were not seen till after they had been brought into hospital. I visited the house where they lived, and noticed several ackee trees bearing fruit plentifully in the yard in which the hut was situated.

CASE 30. C.Y., female, 10 years. Complained of abdominal pain on the evening of February 22nd, and vomited on and off during the day and night of the 23rd. Early on the 24th the child seemed worse, and at 8 a.m. was seized with a convulsion, became comatose and remained in this condition till death, at 10.30 a.m.

The food on the day preceding the illness consisted of yam, salt-beef, bananas, and ackees. The mother knew for certain that the child had ackees on that day, and 'may have had them later, but was not quite sure.'

CASE 31. E.M., female, 25 years. Vomiting on and off, shortly after meals, for a week preceding the date on which I interviewed her, 24th. Her food had consisted of bananas and ackees. She attributed these attacks to 'weakness.'

and on the 23rd she determined to take a larger meal of fish, ackees, and bananas. The vomiting had been much worse since, but she had had no twitchings or fits. When preparing her food, she had always boiled the ingredients up together, but had been careful to throw away the 'pot-water.'

This woman, therefore, has suffered from repeated attacks of vomiting after taking ackees, and a more severe attack had followed a larger meal containing them. She always threw away the water, but some had doubtless been absorbed by the solid food.

The last case occurred at a place called 'Tuckers,' near the district of Granville.

CASE 32. P.M., female, between 8 and 9 years of age. About noon on February 23rd, she was given a meal of fish (herring), bananas, and ackees, all boiled together; but, as she said she did not want it, the ackees were removed and the patient drank some of the 'pot-water' or 'soup.' At 2 p.m. she suddenly vomited, after complaining of abdominal pain. The vomiting ceased by 5 p.m., and the patient passed a fair night. At 6.30 a.m., 24th, she again complained of pain, and vomited, so she was brought to the hospital. No further vomiting occurred, the child felt better the same evening, and appeared quite well again by the following day.

This case is a good example of the toxicity of the 'pot-water.'

A brief consideration of the above series of cases may now be given.

For this purpose they may be grouped in the following manner:

1. In sixteen cases, namely, Nos. 3, 4, 5, 6, 7, 10, 13, 14, 15, 19, 23, 24, 25, 30, 31, and 32, there was a definite history of eating ackee or an extract ('soup' or 'pot-water' made with ackees) at the meal preceding the onset of the illness. If No. 22 be included, and there is almost sufficient evidence to warrant such inclusion in this group, there are seventeen out of the thirty-two in which there is no doubt that the attack followed closely on the ingestion of ackees, or an extract from them.
2. Those cases in which there is sufficient evidence to warrant a strong probability that ackees comprised one of the constituents of the meal prior to the onset of the illness. There are six which would come under this heading, namely, Nos. 2, 8, 9, 12, 20, and 21; or, if No. 22 be taken from Group 1, and placed here, there would be seven.
3. This contains only two cases, Nos. 16 and 17, in which there is some evidence pointing to the fact that ackees were eaten, but not sufficiently strong to warrant their inclusion under the foregoing groups.
4. Lastly, there are seven in which no history was elicited of

the eating of ackees, but it must be noticed that in every instance trees bearing ripe fruit were growing in the yards in which the huts were situated, and amongst the poor people it would be most unlikely that the use of a food which was ready to hand, a food of which they all appear to be fond, and which was then ripe, would be avoided, and that at a time when other articles of food are scarce, or at least relatively expensive. The seven included under this category are Nos. 1, 11, 18, 26, 27, 28, and 29.

Briefly, then, we may say that in none of the thirty-two cases could the eating of ackees shortly before the onset of symptoms be definitely excluded; in sixteen the fact was absolutely certain, in seven more it was almost certain, in two more—giving a total of twenty-five—it was probable, while in the remaining seven it was possible.

The eliciting of a history of ackee-eating is not always easy, for, if the native once gets the idea that there is any suspicion of food or other poisoning, he either becomes stolid and unable (?) to grasp the meaning of the simplest question, or states something quite false with the aim of putting the questioner off the scent. Thus, in the case of No. 18, the patient was badly nourished and lived in a poor hut, but surrounded by ackee trees bearing ripe fruit. The mother stated that the child had been given 'just ordinary food,' and when pressed for details would only reply 'yam and beef,' and tried to make me believe that the family lived practically on these two articles always, and protested most vehemently that they would not think of eating any of the ackees which grew so plentifully at their very door.

Again, 'saltfish' is frequently named as an article of diet in the country districts, and in towns such as Kingston 'saltfish and ackee' is a favourite dish with many. In the country parts, however, 'saltfish' may not be fish at all. I had suspicions of this, because when asked what form of salt fish they had had (where this had been named as part of the meal preceding the illness) there was frequently no answer forthcoming. Dr. Thomson, the District Medical Officer, wrote to me on February 26th a letter, from which I quote:—

'I have since learnt, and I have made enquiries myself, from several of the same class of people as those of Salt Spring and Granville, etc., and found that

the statement is true, viz., that these people are in the habit of adding salt to the ackees and boiling it, and then add it to their food, calling it "salt fish." In many instances when they say they had yam, banana, and *salt fish* for breakfast, the salt-fish they refer to is the *salted ackee*.

26.2.15.

(Signed) GEO. WM. THOMSON, D.M.O.'

I have since obtained independent confirmation of this fact from quite another part of Jamaica, that many of the peasants are in the habit of boiling up their ackees and adding salt, and designating the result 'saltfish.'

People generally in this island are convinced that ackees, under certain conditions are poisonous; among these conditions may be mentioned:

- i. Unopened ackees, and by consequence
- ii. Ackees which have not opened naturally, but have been forced after falling unopened.
- iii. Fruit gathered from a decayed branch.
- iv. Ackees with some soft spot in an otherwise apparently sound fruit.

Among the better classes the ackees are gathered carefully one by one, and none are cooked but such as appear ripe and sound in every respect. Among the poorer people, on the other hand, as exemplified in the districts of Salt Spring, Granville, etc., a boy is sent up the tree to shake the branches; ripe fruit and some unopened and unripe fall together; the former is collected, while the latter is left on the ground. These last in time open, and may then be gathered with the new ripe ackees which fall at the next shaking. Apparently an ackee fruit which has opened in the natural way on the tree, and then is allowed to become over-ripe, or even decayed, is not poisonous (so I am informed: I have not had an opportunity of proving the statement), but an unripe, unopened fruit which becomes 'forced' open, the adult native is suspicious of. Children, naturally, would not make this distinction, hence the great incidence among those of tender age.

Some apparently ripe and wholesome ackees have a soft spot in the otherwise firm, fleshy part of the fruit; whether this is due to primary microbial development, or whether it arises from bruising when shaken from the tree, I cannot say, but the fact remains that if this is noticed the ackee in question is not used for food.

Next, from a careful consideration of the histories of the above

cases, the poison is apparently extracted by boiling the affected fruit with water. Nos. 3, 4, 8, 9, 10, 13, 14, 15 and 24 support this. The bananas, pumpkin, yam and ackee are boiled up together; the parents eat the ackees, while the children partake of the rest of the food, the older ones having more of the solid parts, which naturally have absorbed some of the water, and the younger children are given the 'soup' or 'pot-water.' The degrees of toxicity are varied in such cases, as in the series of the Johnson-Clark family (Nos. 3, 4, 5, 6, and 7), where the adults who ate the ackees were slightly affected, having attacks of vomiting, but recovering; the older child taking the solid part of the residue, with, of course, absorbed watery extract, and dying after thirty-four hours; the younger child, who mainly had the 'soup' or 'pot-water,' died in nineteen hours. The Glenn cases (Nos. 14 and 15), and the Cook-Waite cases (Nos. 8, 9, and 10), are other instances in point.

No. 13, a case of a young girl under three years of age, who only had 'pot-water,' is another example of the toxicity of the extract, death taking place in four to five hours.

Undoubtedly a certain degree of suspicion attaches to the use of the 'pot-water' as an article of diet, for, if the family is small and the food sufficient, this water is thrown away before the meal is taken, or the ackees are boiled separately and the fruit taken out and mixed with the other ingredients—yam, banana, pumpkin, etc.—while the water is cast aside.

Whatever the nature of the toxin, it seems to be rendered inert, partially or completely, by stimulants in some cases. Patients who were seen in quite an early stage, the initial vomiting period, which, in my opinion, is gastric in origin, had the best chance of recovery, the stimulant—rum, ether, whiskey, brandy—being followed by recovery, as in cases 5, 17, 29.

On the other hand, when the secondary vomiting has made its appearance, which I believe to be cerebral (see next section), such stimulation seems to have no beneficial effect at all. However mild the case seems to be, and however good the general condition, I have never yet seen recovery take place in vomiting sickness if convulsions occur, or if consciousness is once lost, and I have notes now of nearly three hundred cases.

So extensive an outbreak as this in the Montego Bay district is

sufficient to make out a strong case inculcating the ackee, since so large a number of patients being attacked in such rapid sequence, giving almost identical histories as regards the question of diet, cannot be ascribed to mere coincidence.

I, therefore, next undertook experimental work in order to test this, and establish whether any connection exists between vomiting sickness and ackee poisoning. This is described in Section VI.

IV. SEMEIOLOGICAL

The description of the symptoms of this disease also has a history. When several different affections were included under the comprehensive term 'vomiting sickness,' certain symptoms were mentioned which are not really part of the condition as we know it now.

No advantage would be gained by recording here the earlier accounts of the symptoms when, as has been shown in Section I, so many conditions were placed under the head of vomiting sickness.

Potter's report, though mentioning a symptom here and there when speaking of individual cases, does not give any general or concise statement of the symptoms usually present.

The first detailed description was given in my report to the Secretary of State in March, 1913 (Scott, 1913). This need not be quoted here in full, as it was later amplified for a paper read in July, 1914, before the British Medical Association at their Annual Meeting. This will be given subsequently.

Seidelin (1913) described the symptomatology at length.

In my paper referred to above (Scott, 1914), I have stated:—

'The following is a brief description of the usual train of symptoms; the patient, almost invariably a child, goes to bed apparently in ordinary health. In a few instances only there may be a history of slight indisposition, a cold in the head, or some loss of appetite, or a tendency to lie down during the day preceding the actual onset. During the night the child wakes up and vomits—perhaps only once, perhaps three or four times—and complains of "feeling ill." After an hour or so he drops off to sleep again, and some three or four hours later again wakens up, complains of pain in the stomach (pain is used as a term for mere discomfort, frequently among the native population), and almost immediately begins again to vomit, usually frothy mucus, occasionally bile-stained, and later, only watery fluid, with, in most instances, little or no effort, unless the stomach is quite empty, when troublesome retching ensues; if, however, food is taken, either solid or liquid, there is, apparently, effortless vomiting.'

'In a very short time, often a matter of a few minutes, convulsions come

on, and there is "stiffness of limbs" and a "drawing back of the head" (as the parents describe it); coma rapidly succeeds and terminates in death. In some there is no stiffness or retraction, but a general limp condition. The total duration is short, the average being about 12 hours, or a little more or less. The most rapid in my experience was 35 minutes. Frequently, therefore, the patient is not seen during life, and the history is both incomplete and unreliable, as it is obtained by questioning the parents, who, in the general perturbation of sudden and fatal illness, have not noticed particular symptoms, and, unless volunteered by them, their statements, either in affirmation or denial of a definite symptom, are of little value, a reply to a leading question often varying with the form of that question.'

'When seen during life the child is usually in the convulsive or comatose stage; the temperature is rarely high, usually 101-102° F., but it may be normal. The pulse-rate is between 90 and 100, often fairly strong; respirations 26 to 30, regular till towards the end, when the Cheyne-Stokes type may appear. Kernig's sign is present in some of the cases, and may be distinctly more marked in one leg than the other; rigidity of the neck muscles is more common than retraction of the head, and this rigidity is often overlooked because the flexion is not attempted in the strictly middle line. Rigidity may be fairly marked, but when the flexion is combined with lateral movement (as is the case where the test is applied with the child lying on its side) the stiffness may be masked, since lateral movement may be comparatively free in spite of distinct rigidity of the neck muscles. The pupils are usually equal, moderately dilated, and, if the coma is not deep, react normally. In a few there is photophobia, and in those retaining consciousness, general irritability and complaints of headache—not always by any means severe—usually frontal, sometimes general. Delirium is, so far as I have seen, quite uncommon; shortly before passing into the comatose state the child may remark it "feels very bad," but does not call attention to any particular symptom, or locate the pain, if complained of, to any particular spot. In cases which do not end fatally, the state of coma is rarely present; there are vomiting, headache, convulsions, with only temporary loss of consciousness, and recovery is almost as rapid as the onset. Within 24 hours a child who has been seriously ill may be sitting up in bed, and in another 24-48 hours is up and about, showing practically no symptoms, except a little pallor, general debility, which soon clears up, and some residual headache of no great severity; while others in the family, who did not seem to be any worse at the time, have passed into a state of coma, and died in a few hours.'

Being struck by certain outstanding differences, I added the following remarks to the above:—

'I think that from a perusal of the above one is justified in saying that we have at least two classes of cases. In one there is a rise of temperature, rigidity of neck, possibly retraction, Kernig's sign, and generally rigidity with tenderness; in the other the temperature is normal or hardly raised at all, there is general limpness of muscles, no retraction, no Kernig's sign, no general rigidity, and, so far as my own experience goes, it has only been in the former (and not in all of these) that a diplococcus has been seen in and isolated from the cerebro-spinal fluid, never from the latter.'

It will be well to consider in greater detail some of the individual symptoms.

Up to the present time I have notes and records of 265 cases reported to me as having suffered from vomiting sickness. These comprise both the classes just mentioned, those with 'meningitis-like' symptoms and those without.

Those cases which showed symptoms of cerebro-spinal meningitis, or the most prominent of them, such as headache, vomiting, retraction of the head and neck, rigidity of muscles, Kernig's sign, and from whose spinal fluid a gram-negative diplococcus was obtained, have amounted to fifty-six in number.

The diplococcus in several instances gave the typical morphological characters and the cultural and sugar reactions of the meningococcus; in some cases, however, as will be seen in Section IV, certain differences were apparent. Putting this bacteriological differentiation question aside for the moment, and looking at the matter merely from the symptomatological aspect, we are justified in saying that the symptoms and course of such cases differ from those of the ordinary vomiting sickness patients and may be put in a separate category. They have, it is true, both been included under the same heading for some years past, and this has largely helped in keeping the condition obscure. The differences may be shown in tabular form:—

<i>Ordinary Vomiting Sickness</i>	<i>Meningitis-like cases</i>
1. No prodromata.	1. May be catarrhal prodromata.
2. No headache.	2. Headache present.
3. Pain not common; when present it is abdominal.	3. In addition to headache there may be general tenderness.
4. General limp condition (between convulsions).	4. Rigidity often.
5. No retraction of head.	5. Head retracted.
6. No Kernig's sign.	6. Kernig's sign present.
7. No recovery if consciousness once lost.	7. Consciousness returning between fits, and even retained during fits if not severe.
8. Rapid course—few hours.	8. Course often more prolonged, though sometimes rapid.
9. Recovery complete, if not fatal in 24 hours or so.	9. Recovery slower.
10. Little or no rise of temperature.	10. Fever present.
11. Spinal fluid usually sterile.	11. Organisms present.

In dealing with the symptoms of true vomiting sickness, these cases with meningeal symptoms should be excluded. They are obviously a type of cerebro-spinal meningitis, or, one ought,

perhaps, rather to say a variety of this disease, as a meningococcus-like organism is found in association with the above symptoms.

Of the remaining 209 cases there are 21 which will require a little consideration as to whether they should be classed as ordinary vomiting sickness cases or as 'meningitis' cases.

CASE 1. D.H., male, 3 years. History incomplete; obtained from parents after child's death. Spinal fluid was turbid and gave a growth of meningococcus-like organisms mixed with a few coliform bacilli. This tends, therefore, more to the 'meningitis' group.

CASE 2. U.W., female, 7 years. History incomplete, 'hiccough and convulsions, with retraction of the head; no vomiting.' Gram-negative diplococci isolated from the spinal fluid, and there were macroscopic signs of meningitis. This fact, coupled with the absence of vomiting, removes it from the true vomiting sickness group and warrants it being included in the other category.

CASE 3. E.G., female, 10 years. No details of history, but post-mortem there was a pearly haziness over the whole surface of the meninges, and an organism giving the morphological and cultural characters of the meningococcus was obtained from the spinal fluid, which flowed freely on making a lumbar puncture. Therefore placed under the second (meningitis) heading.

CASE 4. W.W., male, 2 years 11 months. History very incomplete, but the spinal fluid was turbid, and yielded a growth on nasagar of a Gram-negative diplococcus. This organism gave the reactions of meningococcus, but also rendered mannite acid. Included under group II.

CASE 5. C.W., female, 2½ years. Vomiting, convulsions and coma; Kernig's sign present. Spinal fluid yielded a diplococcus resembling the last.

CASE 6. P.B., female, 4 years. Included under meningitis series because there were (in addition to the vomiting, convulsions and coma) retraction of the head and rigidity of the limbs, and, post mortem, macroscopic appearances of meningitis, but the case could not be absolutely proved because no specimens were sent to me from this case.

CASE 7. I.S., female, 2 years. Similar to the last; no specimens sent, but macroscopically there were signs of meningitis post mortem, and during life there had been fever, photophobia, retraction of the neck, Kernig's sign, and general rigidity of the muscles.

CASE 8. C.B., male, 4 years. Similar history and post mortem changes; no specimens sent.

CASE 9. J.A., male, 9 years. Suffered from headache and vomiting; retraction of neck and Kernig's sign present; fever. This patient was given injections of meningococcus vaccine and recovered.

CASE 10. M.C., female, 3 years 4 months. Exhibited meningitic symptoms—vomiting, rigidity, retraction of the head, etc.—but not proved, because no specimens were sent. The medical man in attendance stated that he was unable to obtain any fluid by lumbar puncture.

CASE 11. E.M., female, 3 years. This is one of the doubtful cases. The history obtainable was not very detailed or reliable. No doctor saw the child until within two hours of death, when it was comatose. There was a history of a cold in the head preceding the onset (? premeningeal catarrh); there were

vomiting, convulsions and coma, but not rigidity nor Kernig's sign when seen by the medical man shortly before death. The spinal fluid, however, yielded a pure growth of the meningococcus. Of such a case, Seidelin states (p. 413), 'Cases of this nature, observed during an epidemic of cerebro-spinal meningitis, would be accepted under this diagnosis without much discussion.' Hence I would include it under heading II.

CASE 12. H.S., female, 3½ years. No history of any sort obtained, the child not being seen till after death. Fluid obtained by lumbar puncture gave a growth of a Gram-negative diplococcus which rendered glucose, maltose, and galactose acid (the last very slightly).

CASE 13. J.G., male, 8 years. Similar to the last.

CASE 14. V.L.C., female, 4 years. No specimens were sent from this patient, but the medical man who attended, stated 'I regarded this as a perfectly typical case of epidemic cerebro-spinal meningitis, with all the classic symptoms An order was given for burial, so I was unable to perform the post-mortem.'

CASE 15. D.D., male, 11 years. This is almost certainly a case of meningitis, but, unfortunately, the culture tubes were all broken up in the post. But the history practically suffices to establish the diagnosis. 'There were frontal headache, chilliness; photophobia, 24 hours later; neck and head retracted, irritability with frequent (effortless) vomiting, convulsions and coma.' Total duration of illness 3 days.

The following six, however, though differing from the usual case of vomiting sickness, would be included under that head rather than under the other, i.e. as a possible variety of vomiting sickness.

CASE 1. B.D., female, 4 years. This is put into a class different from those just considered, because the history was not that of true cerebro-spinal meningitis, and the coccus obtained was atypical in several respects (see later, Bacteriology, Section V). At the same time it is worthy of note that there was diffuse hyperaemia of the meninges, the brain was oedematous, the spinal fluid flowed freely on making a lumbar puncture. Finally, it was one of the cases of which Seidelin spoke as being accepted as suffering from cerebro-spinal meningitis, had they occurred during an epidemic of this disease.

Nevertheless, it was not a typical case of vomiting sickness, because there was no vomiting.

In placing it, one is in doubt whether to regard it as an atypical one of vomiting sickness associated with a peculiar organism (*M. jamaicensis*), or as an atypical case of meningitis associated with an atypical diplococcus.

CASE 2. F.P., female, 3 years. The history of this patient was not very complete; all that could be obtained was that she was suddenly attacked with vomiting, soon succeeded by convulsions, which recurred at frequent intervals till death; and, that the head was 'drawn back'; the spinal fluid flowed freely, and yielded a growth of Gram-negative diplococci, associated with some diphtheroids. The diplococcus had a slight action on glucose only, the rest of the sugars were unaltered.

In this instance, the only differences from typical vomiting sickness were, firstly, the 'drawing back' of the head, and that was not seen by a medical man, but was stated in answer to a question put to the parents after the death of the child; secondly, the organism found. The diplococcus partook more of the nature of the *Micrococcus catarrhalis*, and, both it and the diphtheroid may have

been contaminations, as the cultures were made post mortem, and in the bush, a full twelve hours after death.

CASE 3. M.B., female, 4 years. The history of this patient differs from that of ordinary vomiting sickness. Thus, the fits preceded any vomiting by a considerable interval; there is said to have been retraction of the head, and the vomiting is stated to have continued up to the time of death. The spinal fluid flowed freely when a lumbar puncture was made, and smears from this showed well-marked Gram-negative diplococci, but no growth was obtained on the media used. The presence of these in the smears of the fluid, together with the above history, would be sufficient in an epidemic to class the case as one of cerebro-spinal meningitis; but, as the history was not typical, and, as no growth was obtained, this cannot be said to have been proved, and the case has, therefore, not been included in the meningitic category.

CASE 4. E.A., female, 3 years. This cannot be regarded as a case of true vomiting sickness. Firstly, there was no vomiting at all, but convulsions with 'trismus, opisthotonus, and retraction of the head' (medical officer's statement). The meninges were found to be intensely congested, with dulling at the base; the fluid was not in excess. No specimens were sent, so the nature of the case cannot be further elucidated, and I fail to see why it was reported as one of vomiting sickness at all.

CASE 5. J.E., female, 6 years. Except for a longer duration of illness this patient showed symptoms similar to the last; she lived in the same district. There was no vomiting, and no specimens were sent from the autopsy. The description of the latter was also very meagre, and no positive diagnosis can be arrived at. With the exception of convulsions, none of the usual symptoms of vomiting sickness were present.

CASE 6. C.G., male, 6 years. The history of this case was typical of that of vomiting sickness, but, at the autopsy, it was noticed that there was intense meningeal congestion, the cerebro-spinal fluid was in excess, was uniformly turbid, and there were deposits of lymph on the meningeal surface. Lastly, from the spinal fluid was grown a Gram-negative diplococcus giving acid in glucose, maltose, and galactose. This patient was not seen by a doctor during life; the history may, therefore, be faulty, and the case be one of ordinary cerebro-spinal meningitis, but it has not been included amongst those on account of there being no support for it in the history of symptoms.

Having then briefly considered atypical cases, we may pass on to deal with true vomiting sickness. The characteristic symptoms are: vomiting, convulsions, and coma, with general limpness of muscles, and usually early death. As a rule there is no rise of temperature. In a mild case, or rather in a non-fatal case, there are neither convulsions nor coma, and in a few instances the vomiting (the initial vomiting, at any rate) is absent. To the above symptoms may be added one which is occasionally present, namely pain.

Let us consider the symptoms individually as evidenced in 188 cases of true vomiting sickness.

1. *Pain.* This is not a frequent symptom. The word is often used for mere discomfort, and on no occasion to my knowledge was

acute pain present. The patient was never 'doubled up' with pain, nor was it, as a rule, severe enough to cause the patient to resist or resent palpation.

I can find mention of it in only 41 instances out of the 188. In 33 of these the site of the pain was abdominal or rather epigastric, and this symptom when present always appears to have preceded any others.

In the remaining eight the pain was referred to the head, but in them it appeared subsequently to the vomiting, and was either due to congestion in the secondary (cerebral) stage, or to the strain of vomiting, since to this part was referred the pain in the case of four patients who recovered, and in none of them was it severe.

2. *Vomiting.* Seidelin states (p. 452) that out of 40 cases vomiting occurred in 24; it was absent in 6, whereas in the remaining 10 no definite information was obtained.

In my series of 188 this symptom was in evidence in 162, was absent in 15, and no information was given in the histories of 11 patients. Leaving out these last, we may say that it was present in 162 cases out of 177 of which histories were obtained, that is in over 90 per cent.

(a) The nature of the material vomited was usually at first food, or, if a considerable interval had elapsed since the last meal, then firstly watery matter, later bile-stained. At times the vomiting may be replaced by troublesome retching. If, as is not very common, the vomiting or retching is severe or prolonged, there may be specks of blood in the vomitus. I, personally, have never seen 'black vomit,' though one can readily understand that, since the congestion may lead to the appearance of specks of blood in the vomit, if this is retained, there may be dark specks in it; this has been described, and I have occasionally seen it myself, but never to such an extent as to render the vomit black.

(b) The times at which the vomiting occurs.

In a typical case vomiting takes place at the onset, in fact it may be the very first objective symptom. It is accompanied, as a rule, by considerable effort, and is repeated, it may be two or three times, at short intervals. This is what I term the 'Initial vomiting,' and gives one very distinctly the idea of an effort on the part of the stomach to rid itself of some noxious material.

In cases which recover, this, with possibly abdominal discomfort

(rarely actual pain), is the only symptom. If the effort is prolonged, there may be a little giddiness and headache, such as would ordinarily be produced by vomiting associated with straining.

In cases which terminate fatally, however, after an interval of calm during which there are practically no symptoms, a return of the vomiting occurs, and this time it is of a different character. It is, to a great extent, effortless, and may not be accompanied by any nausea. This vomiting I designate the 'Secondary vomiting,' and it is, in my opinion, cerebral in origin, owing to its character and also because it is usually followed almost at once by other nervous symptoms—convulsions and coma.

Turning to my series of cases, in consideration of the fact that, as already stated, patients who recover do not exhibit this secondary vomiting, we may say that out of the 148 fatal cases in which the vomiting is mentioned, 86 showed this symptom both at the beginning and later before the onset of other cerebral symptoms.

At the same time one or other may be absent. Thus, the initial vomiting only is seen in cases which recover, and the patients never reach the stage when the secondary, cerebral symptoms appear; again, this initial vomiting is, as it were, suppressed in the very rapid and acute cases. There is an attack of vomiting which is so rapidly followed as to be almost accompanied by the convulsions and coma, the entire symptoms being cerebral, owing to rapid absorption of the toxin from an empty stomach, and death may then take place in an hour or even less.

In much rarer instances the secondary attack of vomiting is suppressed; the patient may pass through the initial attack and appear to improve; then, after a considerable (but varying) interval, he is seized with convulsions, passes into the comatose stage and dies.

Lastly, vomiting may be absent; the cerebral symptoms may be the first indications of anything wrong. For example, a child of four years of age was quite well when she ate her dinner at 1 p.m. Two hours later she felt out of sorts and went to lie down. An hour or so afterwards her mother went to awaken her, but could only partially do so; twitching of limbs and slight convulsions came on, and the child lost consciousness altogether and remained comatose till death.

Such cases have been diagnosed, somewhat paradoxically, as 'Vomiting sickness without vomiting,' and I am of opinion that they do occur, though exceedingly rarely. Such a diagnosis, however, could not be made unless (i) true vomiting sickness cases were occurring at the time, (ii) all other causes could be excluded, or (iii) the post-mortem signs, especially microscopical, were those of vomiting sickness (see next section).

The vomiting sickness returns are, I regret to say, unduly swelled during the season at which the disease prevails by reports of deaths as such merely because the child had an attack of vomiting sometime during its final illness, or if death occurs and no adequate cause is found, the case is certified, as already stated, as one of 'vomiting sickness without vomiting,' and the autopsy is thus avoided.

Briefly, to sum up with regard to this symptom:—Initial vomiting only was present in 65 cases, including 34 who recovered; secondary vomiting only in 11; while both initial and secondary occurred in 86 instances.

3. *Convulsions.* Opinions as regards this symptom are a little varied. In some histories where this symptom is mentioned, further enquiry elicited the fact that merely slight twitching movements of the limbs were noticed. In other cases there were definite tonic contractions of muscles, lasting for a few seconds only, while again some were described as clonic. The uncertainty arose often from the fact that the patient was not seen by a medical man during life, and the statements of parents, other relatives, or friends had to be relied on.

Looking over my notes: In the cases which recovered, 'slight twitching' movements occurred in one patient only, a child of four years of age. Everyone has seen slight twitching movements in a child asleep, apparently in ordinary health, or with a little dyspeptic disorder, so I think one may safely say that in no cases which recover are convulsions seen.

In Seidelin's table the only case of recovery in which convulsions are stated to have been present is noted as not being a definite case of vomiting sickness. His observations in this respect, therefore, agree with my own.

In 151 fatal cases convulsions were definitely stated to have been present in 101. But of the 151 no mention, either positive or

negative, as regards this symptom, is made in 24 instances, and in 8 more the point is doubtful, the history being conflicting. Deducting these, we may say that of 119 cases where convulsions are definitely spoken of, they were present in 101, or 84·87 per cent., and absent in the remaining 18, or 15·13 per cent.

4. *Coma*. In 33 instances no mention is made of this, but in all the other fatal cases it was present, and in none of those who recovered.

Seidelin puts a + under the column of 'coma' in three cases which recovered, but from his description of the patients they could hardly be said to be comatose. Thus, the first case was a girl of four years of age, who showed 'slight collapse' after two hours' vomiting; the second, a baby aged two months (eight months is stated in the table, two in the detailed report) who suffered with 'continuous vomiting and collapse', while the third was a boy of twelve years who, when seen after 'vomiting and retching' for many hours, appeared 'weak and drowsy.'

The coma in most of the cases which I have seen was deep; as a rule there was absolute unconsciousness with no conjunctival reflex, though in one or two there was, at an earlier stage of the coma, some irritability when attempts were made to rouse the patient, but this 'cerebral irritation stage' was transitory and soon passed into one of deep coma.

Other nervous symptoms—Kernig's sign, rigidity of neck muscles, rigidity of muscles generally. Apart from 'meningitis' cases, these were rare in patients seen by a medical man during life. Kernig's sign is only mentioned twice, and in one of these the patient recovered and the symptom had disappeared within twenty-four hours of its appearance, which is sufficient to cast some doubt as to its reality. The second was atypical in many respects; in fact, I have a note to the effect that I cannot understand the reason for the diagnosis, except that the patient, a child of five years, died during the vomiting sickness season. It has been included because the case was so reported and has been so entered in the records. The history of symptoms was very meagre: 'sudden onset, convulsions, no vomiting, Kernig's sign present, teeth clenched, temperature 99·6° F.' There is no other statement and no specimens were sent from this case. May not the so-called Kernig's sign noted

here have been part of the general rigidity associated with the 'clenching of teeth and convulsions'?

In three instances only is 'rigidity of neck' stated to have been present, and then only 'during the fits'; there was no rigidity noted in the intervals.

The same remark applies to four out of the five cases in which rigidity of other muscles is mentioned. In the remaining instance the patient was not seen during life, but, on questioning the mother after the child had died, the history given was that 'the mother woke up to find her child unconscious with teeth locked, arms flexed and stiff, hands clenched, legs stiff, and head drawn back. In other words, the child was in a tonic convulsion.

5. *Fever.* This is not a common symptom at all. The temperature in the majority of cases is normal, and a rise above 101° is quite unusual. In one case 102.4° is mentioned, and in two others 101° ; with these exceptions the highest recorded is 99° F. We may take it, therefore, that in ordinary cases of vomiting sickness there is no fever.

This practically disposes of all the symptoms; the pupils are neither unduly dilated nor contracted, and are equal in all which I have seen, and in all those in which the state of the pupils has been noted by others.

Finally, a few remarks on the questions of age, sex, and duration of illness.

Age. A perusal of Tables I and IV appended will show that the disease is to a great extent one of childhood. Babies in arms are not attacked; only two cases occurred under the age of one year. 87, or 44.84 per cent., however, occurred in the first quinquennium; 58, or 29.89 per cent., during the second; 20, or 10.31 per cent., in the third; and only 2, or 1.03 per cent., in the fourth.

The mortality rate is high in all these periods. Thus, of those attacked between the ages of one and five years 85.06 per cent. died; between five and ten years 86.21 per cent.; in the third quinquennium, out of the 20 attacked 15 died (75 per cent.).

Sex. The disease shows practically no predilection for sex amongst those at the susceptible age. Thus, of 145 cases occurring in the first decade of life (74.74 per cent. of the whole) 65, or 44.82 per cent., were males, and 55.18 per cent. females, and the death-rate

is closely similar, namely, 45.96 per cent males and 54.04 per cent. females.

Dividing the ten years into two quinquennial periods, we see that of 87 cases occurring up to the age of five years 35, or 40.23 per cent., were males, and 52, or 59.77 per cent., were females. Of fatal cases during the same period 43.24 per cent. were males and 56.76 per cent. females. Between the ages of five and ten years 58 cases occurred, of which 30 were males and 28 females; 50 died, of whom exactly half were males and half females.

Duration of Illness. In 140 instances the duration of illness was given. The shortest recorded was in a female child of one year, when death took place within half an hour. The average duration of the whole 140 works out at 12.72 hours. Sex has no influence on duration, for, although of those whose duration is given 82 were females and 58 males, the length of illness between time of onset and death (including, when present, the period of calm) works out at 12.5 hours in the case of males and at 12.89 hours in females, a difference of only 23 minutes.

This section, dealing with the symptomatology of the affection may best be summed up by giving a brief description of four different cases:—

1. Symptoms in a mild case.

A girl, P.M., 9 years of age, was given the soup obtained from fish, bananas and ackees boiled together, at noon, on February 28th. Prior to this she was in her usual good health, and nothing was noticed until, at 2 p.m., she complained of abdominal pain, and suddenly vomited. This continued at intervals for three hours, and the child then went to bed and slept well. Early the following morning she again complained of pain, and vomited. A doctor saw her, but, except for slight epigastric pain, a furred tongue, and a subnormal temperature (97.6° F.), there were no abnormal physical signs present. The pain was not severe, since palpation was not resisted. She was given some stimulant mixture containing ether and ammonia. No further vomiting occurred, and recovery was complete by the same evening.

2. Symptoms in a case apparently mild at first but terminating fatally.

P.H., male, aged 3 years. In usual good health until the evening of March 5th, when he was given a meal of vegetable soup. About two hours later he suddenly vomited, although he had made no complaint of pain. He rapidly recovered and seemed quite well on going to bed an hour or so afterwards. He slept well till just before dawn, 6th, when, without any warning, he again vomited, and was very shortly afterwards seized with convulsions; coma supervened, and death took place at 11 a.m.

The total duration from the first onset of vomiting was 16 hours; there was a symptomless intermission of 8 to 10 hours, and death occurred in five hours after the onset of the secondary vomiting.

3. An average case. This corresponds very closely to the last but the interval is one of improvement, not always of absolute cessation of symptoms.

A girl of 6 years of age went to bed at the usual time, apparently in perfect health. Early the next morning, without complaining, she suddenly vomited yellowish, watery matter, and, in the course of the next hour, vomited twice. During the day she felt sick and stayed in the house. She made no definite complaints and took her food when it was brought to her, but was clearly not quite herself. Towards evening she improved and went to bed, and slept well during the night. The next morning, about 6 a.m., without any obvious cause, and without any effort, she vomited frothy and watery material, and, within a few minutes was seized with convulsions and passed into a state of coma. She did not rally at all, and died at 2 p.m.

4. A case in which convulsions were absent.

A child of 12 years of age, with no record of any previous illness, left home in her ordinary health for school, about three miles away. About midday she had a meal, the nature of which was uncertain. She made no complaint until between 3 and 4 p.m., when she stated that she had pains in her stomach and began to vomit. She vomited three times and felt much better. She then started for home. On the way she felt ill and rested now and again, vomiting at intervals. She did not reach home till 7 o'clock. Shortly afterwards, without any convulsions being observed, she passed comparatively rapidly into a state of coma, which deepened till death, some five hours later.

A case of so-called 'Vomiting sickness without vomiting' has already been described (p. 22).

A reasonable interpretation of the symptoms is that some poison is taken, or some substance which acts as a poison after entering the stomach. If the initial vomiting is sufficient to get rid of this substance, no further symptoms occur and recovery rapidly ensues.

If this is not the case, there is an interval—a more or less quiescent period—during which absorption is going on, and then follow symptoms due to the action of the toxin on the higher centres—secondary (cerebral) vomiting, twitching and convulsions, drowsiness and somnolence deepening to coma and death. In rarer instances it is possible that the cerebral symptoms (convulsions, drowsiness, coma) are the first noticeable; there is no preceding vomiting—the so-called 'vomiting sickness without vomiting.'

V. PATHOLOGICAL

I. ANATOMICAL

Prior to the time of Dr. Seidelin's investigation (1913-1914) there had been no systematic detailed description of the morbid anatomy of this affection. Potter's report (1911) gives brief notes of some twenty autopsies, but these comprise little beyond macroscopic appearances.

Seidelin sums up the anatomical aspect of the question briefly thus (p. 394):—'The most constant and most remarkable pathological changes were: fatty metamorphosis of liver, kidneys, and other organs; necrobiotic changes of epithelia in pancreas, kidneys, and liver; swelling and hyperaemia of lymph nodules; hyperaemia of many organs, including the meninges, and a tendency to haemorrhages; widespread oedema of the connective tissues.'

His descriptions of the various organs and tissues are excellent and full of detail, and in this present section I quote largely from his report, as presenting the question in a clear and adequate manner which can hardly be improved upon.

A. *Macroscopic*

(1) *General condition.* There is a popular idea that the disease occurs mostly in emaciated and badly nourished subjects, if not actually confined to such. This is quite erroneous. Seidelin notes 10 out of his 62 cases as emaciated, and one of these was not definitely a case of vomiting sickness, at least there is a ? mark under the heading 'clinical character.' In my series two were reported as 'emaciated' and three others as 'poorly nourished.' Obviously, therefore, the disease is not one which singles out the weak and debilitated.

(2) *Jaundice.* Seidelin mentions this in his table in one instance only, but he has evidently excluded those with 'slight jaundice of sclerae,' for this is mentioned in the detailed accounts of six cases; in one it is stated to have been general, in three slight, and in two confined to the sclerae.

In my series there was 'slight yellow discoloration of the sclerotics' in seven; in another it was more marked but similarly limited. It must be remembered that the sclerotic of the native often shows an apparent yellow discoloration which on closer examination

proves to be a thin layer of fatty deposit, and might be casually mistaken for jaundice.

On the other hand slight jaundice does occur, and the condition of the liver present in many cases is sufficient cause for this.

(3) As regards other external conditions, some district medical officers have described sores and fissures at the angles of the mouth. I have occasionally seen them in vomiting sickness cases, but so seldom that I am inclined to think that they are accidental, due, perhaps, to syphilis or yaws. Personally, I recollect seeing them but twice.

For purposes of description of the internal organs the contents of the head, thorax, and abdomen will be considered in order.

Brain and Spinal Cord and their Meninges

In almost all cases fluid is obtained fairly readily on a lumbar puncture being made. As a rule, the spinal fluid is clear and flows drop by drop; occasionally it has been cloudy, and it may flow in a steady stream as if under considerable pressure.

The meninges of the spinal cord are often hyperaemic, in fact usually so. The cord itself may in some instances show hyperaemia, but quite as often nothing abnormal is detected.

The cerebral dura is in the majority of cases normal in appearance, but may be hyperaemic; the pia mater on the other hand is more often congested, and the vessels may be engorged, especially on the convex surface of the brain.

There is no fibrinous exudation, though a certain degree of serous meningeal exudate is not uncommon; the brain surface may or may not be congested, and the substance also. In some cases, particularly if the convulsions have been severe or prolonged, minute petechial points are seen on section of the brain substance.

The ventricles are not distended, and apparently the fluid is never markedly in excess.

The hypophysis cerebri may share in the general hyperaemia.

Thoracic contents:

The *Thymus* in one of my cases appeared enlarged, but in none of the others was any abnormality seen.

The *Pericardium* often contains a few cubic centimetres of pale, clear fluid, but not in excess.

As regards the *Heart*, in many cases no abnormality is apparent; it is not uncommon, however, to see hyperaemia of the epicardium, sometimes petechiae and even small ecchymoses. These, when present, are more often visible on the ventricular surface near the auriculo-ventricular junction and posteriorly. They are very rarely seen on the interior aspect of the heart muscle.

The myocardium usually appears normal, but at times there are pale, greyish or yellowish streaks, or patches of small extent. In such the consistence may be a little diminished.

The *Thyroid* is normal in appearance.

The *Pleurae* may show a few petechiae and even minute ecchymoses, but in the majority of cases nothing abnormal is seen. Occasionally a few cubic centimetres of straw-coloured fluid are present in the pleural cavities.

The *Larynx* in some is hyperaemic, as is also the trachea. The trachea and bronchi may contain some frothy mucoid secretion, which in a few instances is blood-stained.

The mucous membrane of the bronchioles may show a diffuse catarrhal condition, a frothy mucus escaping on section. Hypostatic congestion of the bases of the lungs is often present, and these organs may appear hyperaemic and oedematous. By no means infrequently, definite small haemorrhagic infarcts are seen scattered in various parts of the lungs.

The bronchial lymph glands may be hyperaemic and slightly enlarged.

Abdominal contents:

The *Peritoneum* in all cases appears normal, there is no sign of inflammation, and no fluid present in the peritoneal cavity.

The *Stomach*. In some cases nothing abnormal is noticed, but in the majority the mucous membrane is congested, especially over the posterior wall and along the lesser curvature; at times there are several petechiae seen, and occasionally small ecchymoses. The contents are usually grumous, mucoid, frothy material; occasionally dark specks are present.

The *Duodenum* presents the same characters as the stomach. If the latter is normal the former as a rule is also; if the stomach is congested a similar hyperaemic state of the duodenal mucous membrane is also found in most instances.

The *Intestines*. Ascarides are present in some cases, and there may be patches of hyperaemia, especially if the worms are numerous. Otherwise the intestinal mucous membrane is apparently not affected.

Spleen. The capsule is smooth and transparent, the consistence of the organ is usually normal, but may be diminished. Petechiae are occasionally visible on the surface, and the follicles may appear prominent on section.

Liver. Capsule smooth and transparent. The colour of the viscus varies; it may be dark purple, or at times is of a reddish-grey colour with pale patches; again, in a few cases it may be uniformly yellowish-grey. The consistence in such is diminished.

Kidneys. Capsule normal and easily detached; the stellate veins may show out well. The surface may be dark and show capillary hyperaemia; in other cases the surface is pale, and dilated stellate veins are prominent. On section, venous and capillary hyperaemia may be seen, but by no means invariably. The cortex and bases of the pyramids may be hyperaemic, while the apices of the latter are pale. There may be minute haemorrhages and yellowish stripes in the cortex, and signs of a diffuse parenchymatous nephritis.

The suprarenals are in a few cases hyperaemic, but usually there is nothing abnormal detected in them.

The *Pancreas* in many instances appears normal, but may be hyperaemic and of diminished consistence.

In four cases reported as vomiting sickness I have met with an acute haemorrhagic condition of this organ.

The mesenteric glands are usually enlarged, and in fully half the cases they are found, on section, to be distinctly hyperaemic.

B. *Microscopical*

The spinal fluid is in most cases normal. In some, after centrifugalisation, the cells contained are seen to be mostly mononuclears; a few polymorphonuclears may be present, and in some cases (the 'meningitic' type, rarely in the true vomiting sickness type) Gram-negative diplococci.

Brain and Spinal Cord. The former often shows a condition of hyperaemia, the latter less commonly. The pia mater may show some oedema and patches of microcellular infiltration.

Heart. The muscle-fibres may show fragmentation, and more rarely minute fat droplets.

Lungs. In parts of the lung tissue taken from the bases there is general congestion; the bronchioles exhibit swelling of the cells of the mucous lining, and shedding of epithelium, the alveoli also contain shed cells. In the infarcted parts the alveoli are filled with red blood corpuscles, leucocytes, and shed epithelium, but no organisms are discoverable.

In a few cases fat droplets have been seen in the epithelial cells.

Stomach. In the majority of cases there is localised hyperaemia of the mucosa, and there may be diffuse microcellular infiltration of the mucosa and submucosa. Less often minute haemorrhages are visible.

The Duodenum exhibits similar changes, but less frequently. There may be hyperplasia of the lymphoid follicles with slight microcellular infiltration and patches of hyperaemia. The glands of Brunner are at times prominent.

Liver. In most there is irregularly distributed capillary hyperaemia, and there may be microcellular infiltration of the periportal tissue. In the majority of cases the cells of the parenchyma show a fatty change, which varies between the very slight and the intense. In some cases this fatty change appears to be more marked in the neighbourhood of the portal vessels, in others it is fairly equally distributed over all zones of the lobule.

Spleen. In many cases there is little or nothing abnormal to be made out. There may be diffuse hyperaemia and the follicles may show necrobiotic changes. Both these conditions may be of irregular distribution.

Kidneys. In most instances there is renal affection. Hyperaemia of varied degree is usually found; this may, in parts, be intense and small extravasations occur. The stroma in some appears to be oedematous and even a little increased, and there is slight microcellular infiltration. The glomeruli may be retracted, and in the spaces between them and Bowman's capsule coagulated serum is seen. In other cases the glomeruli may be distended and hyperaemic. The epithelium of the convoluted tubules and of the ascending loops of Henle shows necrobiotic changes in some cases with granularity and vacuolization and fatty changes, especially at the basal portion of the cells. The nuclei may show karyolysis.

The adrenals in a few instances showed considerable hyperaemia, and, rarely, some vacuolization of the cells.

Pancreas. Necrobiosis of the cells, variable in degree and extent, is common; the cell limits in these cases are badly defined and the nuclei stain poorly. Langerhans' islets are usually (but not always) well preserved and definite.

The limits between the well preserved and the necrobiotic cells may be quite sharp, cells of the two types being seen sometimes side by side in the same lobule or even in the same alveolus.

Sometimes irregular infiltrating haemorrhages are seen, and in many cases the epithelial cells contain several small fat-droplets.

Lymph Glands (especially mesenteric) show a diffuse hyperaemia, patches of necrobiosis, and moderate oedema of stroma.

The *Urine* is, in the majority of cases, plentiful. Possibly, as Seidelin thinks, excreted prior to the onset of the final illness. It is usually normal—clear, acid in reaction, without deposit on centrifuging. But in some there is a little albumen, a few granular and hyaline casts, and occasionally some red blood corpuscles. No organisms are seen.

II. BACTERIOLOGICAL

Upon this subject I have very little to say, because investigations which have been carried out during the last two years make me incline more and more to the opinion that the disease has no bacteriology. I mean by this that the organisms which have been found in some of the patients described as suffering from vomiting sickness are not causative.

The bacteriology of the affection is largely of historical interest.

Dr. Seidelin's account on pages 458-465 of his report is excellent, and well worth studying by any who wish to trace the stages through which the descriptions of this disease have passed.

As I have stated in the previous section, true vomiting sickness cases can usually be differentiated clinically from the 'meningitis-like' ones in which the diplococci have usually been found. In the season of 1913-14 out of twenty cases I obtained the diplococcus only twice, and in one of these the cultural reactions differed from those of the meningococcus. In another the coccus was atypical in tending to grow in chains and also in producing acid in mannite and raffinose.

In one case only amongst those seen by me in the Montego Bay outbreak this year were diplococci seen in the spinal fluid, and in this instance no growth was obtained. They were visible only in a smear of the fluid after centrifugalisation; cultural attempts failed completely.

My opinion, the result of prolonged investigations, is this:— If we set aside true meningitis cases in which the meningococcus is found, such as the 'Peart' series in 1912, which are few; and if we set aside also cases with anomalous symptoms which have been previously included under the comprehensive term of vomiting sickness, and of which I have spoken in the last section; then the organisms found in the remainder—the true vomiting sickness cases proper—are accidental concomitants, or at the most are part causes only; and even of this latter idea I have grave doubts.

My reason for this are three:—

(i) In by far the majority of cases no such organisms are present at all.

(ii) When cultivated and injected in a living state and in large doses into laboratory animals—guinea-pigs, rabbits, Belgian hares—no untoward effects were discovered. Inoculations were made subcutaneously, intraperitoneally, intravenously, and intracardially.

(iii) The organisms showed extensive variations. They varied as regards—(a) Form: sometimes occurring in groups, sometimes tending to chain formation; (b) Size: sometimes as small as the gonococcus, say, 6μ , at other times more than double this; (c) Staining reactions: sometimes readily decolorized by Gram's method, at other times with considerable difficulty; (d) Sugar reactions: galactose is sometimes rendered acid, at other times not; lactose, saccharose, mannite, raffinose are in some cases acted upon.

As Seidelin very pertinently remarks (p. 464): 'Experience has shown in several diseases that a germ which has for some time been regarded as a pathogenic one, has, on close investigation, been reduced to a secondary position as representing only a complicating infection.'

With respect to vomiting sickness I would fully endorse this, and, perhaps, go even a step further and say that vomiting sickness

pure and simple is not, in my opinion, a bacterial affection, but an intoxication; that the organisms found in the spinal fluid are either accidental and non-pathogenic, or at most may intensify the nervous symptoms by causing an increase in the amount, and consequently in the pressure, of the cerebro-spinal fluid.

The diplococcus is much more often absent than present even in typical cases, and in by far the majority of instances cultural attempts, as well as direct examination of the blood or spinal fluid, yield only negative results.

VI. EXPERIMENTAL

My own work in this connection will be described in detail later in this section. When I started investigating the question of ackee poisoning early in the present year (1915) I was unaware that any work had previously been carried out on the subject. I was informed, however, by Mr. Robert Simmons, F.I.C., the Deputy Island Chemist of Jamaica, that a predecessor of his, Mr. J. J. Bowry, had undertaken an investigation into the nature of the ackee poison as long ago as 1886. Mr. Simmons very kindly looked up the notes of this enquiry and sent me an abstract of them, and also a brief tabular statement of twelve deaths which followed the eating of the fruit of the ackee (*Blighia sapida*).

He also gave me the following letter which probably led to Bowry's investigations being undertaken:—

‘MONTEGO BAY,
February 26th, 1886.

‘DEAR MR. BOWRY,

‘I have, with great interest, read your favour of the 25th inst., along with enclosed extract from a medical man, giving particulars of the deaths of several children in Vere.

‘After a careful consideration of all the facts I cannot arrive at any other conclusion than the cases there mentioned were those of narcotic poison, and the particular poison in this case, I feel confident, was that of the “Ackee,” as the symptoms exactly tallies (*sic*) with my own experience of the poisonous action of this fruit. If you will go to a little further trouble, and have enquiries made, I think you will find that these children had been playing probably the day before under ackee trees, somewhere in the district, the probable inference is that they picked up portions of the decayed fruit and ate it. A great many times this condition of affairs have (*sic*) been brought to my notice, in the majority of the cases I have not been able to prove that they did actually eat; but, the history you always get is that they were playing under the tree, and, more out of mischief, eat small portions of the decayed fruit that had been for some days on the ground.

At a period of about 12 hours after the action of the poison commences, exactly as described in the extract from the letter you sent me, and always ends fatally. In my experience I have never seen a case of recovery, the prominent symptom being gradually increasing coma.

'I will write you again in a few days.

I remain, Dear Sir,

Yours very truly,

ALEX. McCATTY.'

I have quoted the letter verbatim. (H. H. S.)

Bowry's experiments in ackee poisoning were undertaken to discover, as a chemist, the nature of indigenous poisons in Jamaica. There is nothing whatever to indicate that he suspected any connection between ackees and vomiting sickness. This connection dates only from my investigations this year at Montego Bay.

Returning to Bowry's results, Mr. Simmons states: 'He (i.e. Bowry) ascertained that when the white portion of the ackee 'was soft it was poisonous; that an unripe or decayed ackee was 'poisonous. The edible portion of ackees taken from a broken 'bough was decaying, whether the fruits were open or still closed. 'He found that ackee fruits with no, or very small, or undeveloped 'seeds were those specially prone to develop the poison.'

In Bowry's own words: 'In each case which has come under my 'notice of ackee fruits being poisonous, it has been fruit without well- 'formed seeds which have been deadly. Not that fruits with 'abortive seeds are necessarily poisonous; when ripe and perfectly 'fresh, I believe them to be as wholesome as those with perfect seeds, 'indeed by actual experiments I have found such ackees to be 'wholesome. But these seedless fruits are apt to become over-ripe, 'stale, even decayed, without opening.

'It would appear that the decay which takes place in the 'unopened fruit results in rendering it deadly, as decay in open 'fruit does not. When there are well-formed seeds in a fruit it must 'open before decay begins, while seedless fruits will often remain 'closed until the edible portion has become quite soft and poisonous.'

The attached table was compiled from notes of cases of undoubted ackee poisoning with which Bowry dealt, and in which specimens of the ackees supposed to have caused death were forwarded to him and proved by him to be poisonous. He obtained extracts from suspected ackees and proved their toxicity on various animals, but he never succeeded in obtaining the same poisonous extract from the viscera of a person poisoned by ackee.

To quote again Bowry's own words : ' There is no known method of recognising poisonous ackees after they have been eaten, even if any remain in the stomach after the vomiting which poisonous ackees cause, for persistent vomiting is the most marked symptom in cases of ackee poisoning.'

He draws attention to the increased difficulty of attempting to recognize the poison in viscera owing to the stomach contents being lost by vomiting. In one case he noted that the fruits from a 'broken bough' were undergoing a peculiar form of decay, the edible portion being soft and slightly discoloured, and the membrane, which is usually red and tough in good fruit, was broken and soft in the shrivelled ackees.

An extract prepared from the edible portion of these fruits proved poisonous to mice and cats, with symptoms resembling those seen in the persons who died. A similarly prepared extract from fresh ackees bought in the market proved harmless.

He found that the edible portion of wholesome ackees, minced and allowed to stand for a week until mouldy and rotten, did *not* yield a poisonous extract, but that some of the same ackees similarly treated for the same time, with the addition of a small quantity of the ackees from the broken bough, yielded a very poisonous extract.

This showed that it was some specific change which led to the production of the poison, and not ordinary decay. He held the view, therefore, that the poison was produced by some enzymic or catalytic action.

As regards tests, he writes : ' No tests other than physiological are yet known for the ackee poison, and, as vomiting is a most marked symptom, all poison still unabsorbed into the system is entirely removed from the stomach before death takes place.'

He also incidentally refers to the difficulty in obtaining reliable information as to symptoms, their nature and time of appearance, and the interval of time elapsing before death.

In commenting on this, he says : ' Immediately the Police take a case up the friends and relatives of the deceased become very unwilling to give any information, fearing that they may be charged with some offence in connection with the occurrence. They contradict each other, every item of importance has to be dragged

'from them, and no satisfactory conclusion can be drawn from their statements. But for this fear of ulterior consequences many cases which now remain shrouded in mystery would become plain.

'I have known the relatives of a deceased child try to make out that it could not have eaten ackees, although eventually they have admitted there was an ackee tree in the yard from which the child could have obtained fruit, and I have found the fruit to be of a very suspicious character.'

The previous sections of this paper have shown how frequently I have met with the same difficulty in eliciting true statements on these points from the relatives.

In view of my own findings, Bowry makes two other remarks of interest. The first is that in his experience the history of ackee poisoning showed that children constituted the greater number of victims, 'probably because they are not acquainted with the conditions under which ackees are considered by adults to be dangerous, and so pick them unripe, off broken limbs and from the ground, and eat them with fatal results.' The second is that he is 'disposed to think that many deaths which have been set down to ackee were really due to cerebro-spinal fever, and that it is a mere unconnected coincidence that the climatic influences which give rise to the disease occur during the ackee season.'

When one considers that, in the light of my remarks in previous sections of this paper, I arrived at the same conclusion—that vomiting sickness and cerebro-spinal meningitis cases have been confused and included together in the same category—the statement of Bowry's gains increased interest, and had I known of it, I should have been saved months (even years) of work.

His table, attached, is of interest in that two-thirds of the cases occurred in children within the first decade as compared with 74 per cent. of my series of vomiting sickness patients. The symptoms are similar, namely, vomiting, convulsions, coma and death. The duration of illness he estimates a few hours longer than in my series, but it must be noted that he definitely remarks on the difficulty he found in obtaining reliable information as to the interval of time elapsing before death took place.

The most important defect in the table is the absence of any description of the condition of the organs and tissues,

Age	Symptoms	Death in	Certified cause of death	POST-MORTEM RESULTS						Remarks
				Brain	Stomach	Stomach contents	Intestines	Liver	Kidneys	
4	Severe vomiting	13 hours	1. Acute irritant poison 2. Gastritis	Intensely congested	Mucous membrane irritated and congested	Frothy, thin, dirty liquid	—	—	—	Deceased and his brother (aged 7) ate an unopened ackee
8	Persistent vomiting	13 hours	Syncope	—	—	—	—	—	—	Vomited up portions of ackees
7	Convulsions	16 hours	1. Ackee poisoning 2. Syncope	Congested	Normal	Thin fluid	—	Swollen	Congested	Ate raw ackees
14	Vomiting, collapse, coma	About 24 hours	1. Ackee, ptomaines? 2. Syncope, asphyxia	—	Normal	Yellow fluid	Normal	Normal	Normal	Ate freely of ackee soup. The father also partook of some, was very ill afterwards, but recovered
13	"	"	"	—	"	"	"	"	"	
2	Coma	15 hours	—	—	—	—	—	—	—	Ate ackees
2	Vomiting, headache, fits	About 42 hours	1. Poison from eating unripe ackee 2. Convulsions	—	—	—	Distended	Enlarged	Congested	All ate from a heap of decayed ackees which had been lying in the yard for about a week
4	"	"	"	—	—	—	"	"	"	
6	"	"	"	—	—	—	"	"	"	
11	"	"	"	—	—	—	"	"	"	
Child	Fits	14 hours	—	Congested	Normal	Thin gruel-like liquid	Normal	—	Congested	Ate uncooked ackees
29	Vomiting and severe purging	72 hours	Ackee poisoning	—	Not inflamed	Thick matter	Distended	Enlarged	Healthy	Picked ackees, cooked them next day, ate them and gave some to her child, aged 4; child became ill, but recovered. Deceased was a weak woman

macroscopic or microscopic. The reason for this was, that there was no pathologist in the island until many years later.

Seidelin (1913) mentions briefly (pp. 460-462) some experimental work with some of the diplococci isolated from cases seen by him, but they did not appear to have any pathogenic properties as regards the animals he employed.

From 1886 to the present year (an interval of 29 years), when my investigations into the Montego Bay outbreak, recorded in Section III, led me to suspect ackee poisoning as the cause of the mysterious vomiting sickness, the subject appears to have been completely dropped, in abeyance if not altogether forgotten, except for the two or three casual remarks to which I have called attention when describing the history of the disease, in Section I.*

At my request, Mr. Simmons kindly prepared some ackee extracts made from fruits with undeveloped seeds and (*a*) firm arilli, (*b*) decayed arilli, using absolute alcohol, amyl alcohol, ether, and petroleum ether.

The method of preparation of each was as follows:—

(1) *Ackees with imperfect seeds and firm arilli.*

(A) The macerated arilli and placenta were treated with absolute alcohol in the cold for two days. After filtration, the solvent, which was of an orange colour, was evaporated under reduced pressure at 60° C. To the residue more absolute alcohol was added, and the precipitate formed removed by filtration. The filtrate was evaporated under reduced pressure at room temperature. The residue was dissolved in water, filtered, and tubed.

(B) The arilli and placenta were allowed to stand under reduced pressure for two days, at the end of which time the arilli had become soft and mouldy. The filtered absolute alcohol extract was greenish in colour, and addition of water produced a milky appearance. The weak alcoholic liquid was extracted with amyl alcohol, the latter removed, and the aqueous portion treated with petroleum ether to remove traces of the amyl alcohol. The aqueous portion was then evaporated, taken up with water again, filtered and tubed.

* Section I, dealing with the history of this disease has been abridged; the statements referred to may be seen in the official reports of the Island Medical Department from which the (unabridged) history was compiled.—H. H. S.

(2) *Ackees with undeveloped seeds and decayed arilli*

(C) An absolute alcohol extract made in the cold was gently evaporated, and left a brown resin which was insoluble in hot absolute alcohol, but soluble in water. The resin was removed by filtration, and the liquid concentrated. The residue, dissolved in water, was treated with basic lead acetate, excess of lead removed by hydrogen sulphide, and the filtrate evaporated. The residue obtained was still of a brown colour, and contained minute acicular and cubic crystals. The aqueous solution of this residue was tubed.

(D) The resin above was dissolved in water and tubed.

(E) A hot absolute alcohol extract on cooling deposited minute crystals which re-dissolved on heating. The evaporated extract treated with water resulted in the separation of an oil. The filtrate from this was evaporated to a smaller bulk, and tubed.

(F) An extract of ackees dried *in vacuo* was made with petroleum ether; the evaporated extract left a considerable quantity of yellow oil. The residue was treated with water, the oil removed, and the solution concentrated and treated with absolute alcohol. The precipitated matters were filtered off, the liquid evaporated, taken up with water, and tubed.

(G) Similar to the last, but using ether instead of petroleum ether.

I tested these by intragastric administration to guinea-pigs, rabbits, and Belgian hares. The results, however, were indefinite; but the experiments are mentioned because I think that one can learn something from the very fact of their failing.

In my opinion the failure may be attributed to one or other of three causes:—

(i) The amounts of extracts made were small. Mr. Simmons was very busy at the time, and the preparations had to be carried out in addition to his ordinary routine work, which is heavy.

(ii) The extracts were all made with alcohol or ether as the primary menstruum. Judging from the results of early treatment, if stimulant in the form of alcohol (rum) or ether is given, recovery ensues. This is very probably due to precipitation of the poison rendering it non-absorbable if not inert (see later, Section VII). If this is so, the filtrate from treatment of the ackees with alcohol would very likely not contain the poison.

(iii) The animals employed were all herbivora. I could not at the time obtain carnivora—kittens, pups, etc. Subsequent experiments have tended to show that herbivora are probably not susceptible to the poison, or, to say the least, are relatively insusceptible.

I next proceeded on a different track. It is difficult to understand why I did not try the following method first, as it would seem to be the one dictated by commonsense.

The end in view was to establish whether any connection existed between vomiting sickness and ackee poisoning, as appeared likely from the investigations into the Montego Bay outbreak.

In order to simulate as closely as possible the conditions under which, by this hypothesis, cases of vomiting sickness occur, I obtained some ackees which appeared good except for the fact that they were 'unopened' or had been forced open after being gathered.

These 'suspected' ackees were then boiled with water in the same way as that in which the fruit was used in the Montego Bay and outlying districts of Salt Spring, Granville, etc., in making the 'soup' or 'pot-water.' This ackee extract, as it may be called, was then filtered and used for experimental administration to animals. The extract thus obtained is a liquid of the colour of weak tea or thick soup with a layer of oily matter like melted butter floating on the surface. The animals employed, namely, three kittens and one dog, were fed with the extract, thus carrying out the conditions under which the pot-water is taken in the country districts.

The details of each of the four will be given:—

Kitten 1. To all appearances perfectly healthy, five weeks old, weighing 500 grammes.

On March 28th, 1915, at 11 a.m., a dose of 5 c.c. of the watery extract was administered intragastrically. At 11.40 a.m. it suddenly vomited yellowish-brown watery matter, and seemed to be heavy and dull for half an hour or so, but was not comatose. During the afternoon it recovered, apparently completely, and by 4 p.m. was quite lively. The only other food given was fresh milk.

The next day, March 29th, it seemed to be quite well and had taken the milk provided. At 12 noon 10 c.c. of the same extract was administered in the same way. At 1 p.m. vomiting set in, and this was repeated during the next half-hour, after which the kitten

again improved and began to play about. At 3 p.m. another 10 c.c. was given. Vomiting again ensued, this time 45 minutes after administration, the animal became dull and somnolent, and could only be roused with difficulty; it could not walk but lay limply. Between 4 and 5 p.m. this somnolence deepened to coma, and death took place the same evening. No convulsions were observed. The total amount given was about equivalent to the extract obtained from one ackee.

At the post-mortem on the 30th the *liver* was noticed to be paler than normal, and the lighter parts were in patches; the *kidneys* were congested; the *bladder* was about half full of clear urine. The *stomach* showed a patch of slight congestion, no extravasation or petechiae. The *lungs* showed an infarct in the left, occupying about one-third to one-fourth of the lower lobe. No enlarged lymphatic glands were noticed. The vessels of the *meninges* were congested.

Histological examination yielded the following appearances:—

Liver: General engorgement, capillary and venous, hepatic and portal; in parts distinct extravasations among liver cells. Some oedema of stroma. Liver cells granular with vacuoles, sometimes four or five small, rarely very large; generally distributed, not more at periphery than towards the centre of the lobules. Nuclei generally stain well, but some degree of necrobiosis in parts showing the largest or most numerous vacuoles. Small microcellular infiltrations here and there.

With Flemming: Vacuoles (fat) in some parts much larger than in others, and in these the nuclei stain badly, in some cases being barely visible, and others showing karyolysis. Some cells, again, contain two or three vacuoles of considerable size, but nucleus is well defined; others contain several, in fact, the protoplasm is largely vacuolated, and the nuclei are fragmented and stain poorly.

Kidney: Hyperaemia, cortical venules distended. Some Malpighian tufts have dropped out, and, in their place, are red blood corpuscles; others show a lining of blood just within Bowman's capsule, and the Malpighian body has shrunk from the capsule. In nearly all there is a lining of fibrin or corpuscular debris between the glomerulus and the membrane. In the convoluted tubules the epithelial cell limits are badly defined, but the nuclei mostly stain well; the protoplasm is granular and the tubules often contain granular debris, which may be corpuscular detritus. In parts there is considerable necrobiosis, epithelial cell limits not definable, and nuclei badly stained. Some of the cells also show small fat granules at their bases, while occasionally the epithelium is distinctly vacuolated. In the straight tubules many of the nuclei stain poorly; the ascending limbs of Henle's loops contain debris similar to that mentioned above. There is a condition of general congestion, and in one or two spots small extravasations. No increase of stroma.

Pancreas: Vessels in interlobular tissue engorged. The epithelium shows some degree of necrobiosis, but not very marked; the islets of Langerhans show up well. Epithelial degeneration varies in different parts of the section; in some the cells appear almost normal, but in others they are granular, cloudy, and the

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Pancreas: Vessels in interlobular tissue engorged. The epithelium shows some degree of necrobiosis, but not very marked; the islets of Langerhans show up well. Epithelial degeneration varies in different parts of the section; in some the cells appear almost normal, but in others they are granular, cloudy, and the

nuclei stain badly. In sections from another part of the gland the cells are vacuolated, and the stroma appears oedematous. No haemorrhages are seen.

Stomach : Nothing abnormal seen microscopically.

Spleen : Malpighian bodies prominent. Vessels are congested. There is oedema of stroma, possibly some increase, with small-celled infiltration in parts. In patches are areas of cells with poorly staining nuclei, and in these parts is also seen some débris, strongly suggestive of small extravasations, though definite corpuscles cannot be made out.

Lung : Intense congestion in parts, wedge-shaped as infarcts, and here the alveoli are largely occupied by fibrin, but some contain corpuscles. Alveolar walls here and there are broken down, so that spaces equivalent to four or five alveoli are filled with fibrinous débris, intermixed with which are occasional leucocytes and epithelial cells.

Giemsa stained sections show the presence, here and there, in the congested patch of organisms, diplococci and others (see later, after description of Kitten III). Flemming sections show no fat.

Heart muscle : Fragmentation of the fibres, and, in parts, some of the nuclei stain badly, while the fibres show coarse longitudinal striation with obscured transverse. Most of the fibres and nuclei stain normally. In two or three situations there are minute extravasations of blood between rows of fibres.

Thymus : Congestion of vessels, some oedema of stroma and minute extravasations are seen in a few places. Hassall's corpuscles well shown.

Cerebrum : Vessels of meninges congested ; also small vessels of the interior. Nothing else abnormal noticed. Nuclei generally stain well, and are normally situated in the cell. This tissue was not taken in Flemming's solution.

It will thus be seen that the characteristic symptoms of the so-called vomiting sickness appeared about one hour or so after the administration of filtered watery extract of ackee. After a small initial dose there was vomiting and rapid recovery ; after a larger dose more severe vomiting, and after a third a repetition of the vomiting with drowsiness progressing to coma and death.

The post-mortem findings were typical of those seen in cases of human vomiting sickness, except that some micro-organisms (pneumococci) were found in the infarcted part of the lung.

These have not been mentioned in human cases in which infarction occurred, perhaps they were not present, possibly they were overlooked. I believe they were accidental in this case (see p. 49).

Kitten II. Quite healthy, six weeks old, weight 555 grammes. On April 20th, at 11 a.m., 5 c.c. of freshly prepared watery extract of unopened ackees was administered intragastrically. Within the next hour repeated vomiting occurred and the kitten was inclined to lie down. By 1 p.m. it was again lively and running about, and to all appearances had quite recovered. Some fresh milk was given.

At 2 p.m. another 7 c.c. of the extract was administered, and then followed the same symptom of vomiting, but more severe than after the first dose. The animal was drowsy, almost somnolent, but could be roused. Some milk was left for food during the night, and by the following morning the kitten appeared well again and was playful.

At 11 a.m. on the 21st, 10 c.c. of the same extract was administered; vomiting set in at 11.50 and the attacks were repeated; with intervals of improvement in the general state. At 2 p.m. another dose, the same amount as the last (10 c.c.) was given, and at 3 p.m. the vomiting was again severe, and the animal was very drowsy, the head nodding with sleep, the kitten frequently rolling over on to its side as with muscular relaxation in sleep, and then temporarily rousing up. 15 c.c. was administered at 4.10 p.m. and the animal died the same evening. The equivalent of about two ackees was given in all.

Autopsy at 10 a.m., April 22nd. *Liver* pale, possibly enlarged. The *kidneys* were congested, the *bladder* full of clear urine which on examination was found to contain albumen in small quantity, and after centrifugalisation a few epithelial casts and some red blood corpuscles were seen. The *Right Lung* contained an infarct occupying about one-third of the upper lobe; the *stomach* showed a little congestion and a few petechiae. The vessels of the *meninges* and the cortex cerebri were congested.

Histological examination of the various organs revealed the following conditions:—

Liver: Veins and capillaries congested, hepatic as much as, if not more than the portal. Capillaries between the rows of cells are engorged in parts, and extravasations may occasionally be seen between the cells. The cells are vacuolated and stain badly; vacuoles larger and more distinct towards the periphery than towards the intralobular vein, where the cells are more granular and vacuoles, if present, are smaller. Flemming sections show well-marked fat droplets, but the fluid has, unfortunately, not penetrated through the tissue, and the changes, therefore, are more marked at the periphery of the section.

Kidney: Vessels are congested, and adjacent to the glomeruli may be seen collections of red blood corpuscles. The glomeruli appear to be swollen, but, at the same time, do not entirely fill the spaces, and the intervals are occupied by blood corpuscles and fibrinous debris, while here and there glomeruli appear to have dropped out, and their places are occupied by blood. The nuclei of the glomeruli on the whole stain well. The convoluted tubules show granularity of epithelium; the cell limits are ill-defined, and some of them show small vacuoles at the base, but the majority of the nuclei have taken the stain well. The canals are often filled with granular material.

Flemming sections show considerable fatty degeneration of the epithelium of the convoluted tubules and the ascending tubes of the loops of Henle.

The stroma is more prominent in places, owing to the poor staining of the adjacent tubular epithelium. In many situations the stroma appears to be increased, but is not oedematous, and the nuclei stain well. In some of these parts a sort of mosaic pattern is produced owing to disappearance of the tubular epithelium, the place of the latter being taken by blood, and, where the epithelium remains, the tubules give the impression of being contracted by pressure of the stroma, and such tubules may be seen to contain corpuscles in their lumens.

Pancreas: Epithelium granular, but nuclei stain well; islets of Langerhans prominent. Some of the cells contain fat droplets (Flemming) especially at the periphery of the section. From the appearance of the cells those in the interior of the section would seem to contain fat also, but, unfortunately, the solution has failed to penetrate deeply. The vessels are engorged, the stroma is oedematous, and some degree of necrobiosis is present. No haemorrhages are seen.

Spleen: Stroma appears to be somewhat in excess of the normal; possibly it is oedematous, but the nuclei stain well. There are fairly extensive extravasations scattered through the section. Where the fibrinous and blood débris are greatest the cells appear necrotic and the nuclei stain poorly.

Stomach: Mucous membrane congested, and petechiae present near the cardia. Microscopically, there is an effusion of blood on the surface and amongst the gastric glands at one part of the section.

Lung: Vessels in parts congested; alveoli, on the whole normal, but, in places, some contain granular débris and shed epithelium. At one situation is a well-marked infarct, typically wedge-shaped and fading rather abruptly through a moderately congested zone to healthy lung tissue. No organisms of any kind can be seen; sections were stained for this purpose by Giemsa, by the Eosin-Gram-Weigert, and by the Picro-carmin-Gram methods.

Heart-muscle: Vessels generally congested, some engorged; the fibres are fragmented, minute extravasations are seen here and there. The nuclei generally stain well, and the striation of fibres is well preserved. Nothing abnormal otherwise; no granularity or vacuolization of fibres. No fat droplets seen in sections treated by Flemming.

Thymus: Vessels full of blood; small extravasations scattered through the gland tissue. Hassall's corpuscles well seen. The nuclei on the whole stain well, but at the sites of the extravasations and in certain lobules are areas of cells staining badly.

Cerebrum: Vessels congested, and in places there is a slight microcellular infiltration of the pia, but these are not very marked nor are they numerous. The nerve cells stain well and the nuclei are distinct almost throughout, but karyolysis in individual cells is not infrequent. This tissue was, unfortunately, not taken in Flemming.

Cerebellum: Shows the same state of things; except for localized small masses of microcellular infiltration of the pia and some oedema, with congestion of the vessels, nothing abnormal is detected.

Kitten III. Six and a half weeks old, quite healthy, weighing 585 grammes.

At 12 noon on April 23rd, 8 c.c. of fresh extract, prepared as before, was administered. At 12.45 p.m. the kitten started to vomit, and did so three times between this and 3 p.m. It seemed inclined

to lie down before the attacks of vomiting, and each time recovered partially and walked about the room. By 4 p.m. it appeared quite well and was playful. The following day, April 24th, there were no signs of disease, in fact the animal was fairly lively. At 10.45 a.m. 20 c.c. extract was given. For 45 to 50 minutes there was no apparent effect; it then became restless and continually lay down; vomiting occurred four times between 11.30 a.m. and 1 p.m. Drowsiness supervened and the animal could only be roused with difficulty. On attempting to walk it was very unsteady. At 1 p.m. another similar dose was given; vomiting ensued almost immediately (very little of this last dose can have been retained) and in a quarter of an hour the animal became comatose, and died at 1.35 p.m. There were no convulsions. The extract from two ackees was given altogether.

Autopsy at 1.50 p.m. The *lungs* showed minute patches of congestion but no definite infarcts. The *stomach* was contracted and contained some of the extract with small lumps (curdled milk; the food throughout had consisted of fresh milk only). The *liver* was macroscopically normal, possibly a little paler in patches. *Kidneys* showed surface veins congested, and there was also some congestion on section. The vessels of the *brain* were full, as in the previous cases.

Histologically:—

Liver: Vessels engorged, portal as well as, but possibly a trifle less than the intralobular; extravasations in several situations, especially towards the centre of the lobules, and there were capillary haemorrhages between the rows of liver cells. These cells showed marked vacuolization, and this condition was more noticeable towards the portal region of the lobules. Some sections showed extensive haemorrhage and destruction of liver tissue. Cell-nuclei as a rule stained well, but many showed some degree of karyolysis.

Flemming fixed sections showed a wide-spread fatty change, often as many as twenty or more black droplets in a cell, often only five or six, but larger. The fat is fairly evenly distributed, but in some lobules the periphery is perhaps a little more affected.

Kidney: Capsule not thickened; subcapsular and cortical vessels very congested and distended with blood. Small extravasations in cortex and parts of medulla. Glomeruli are swollen, but in most instances there is a space between the glomerular tuft and Bowman's capsule, and in some cases this space contains fibrinous and corpuscular debris. The convoluted tubules show swelling of the epithelium, with granularity, and the lumens are, many of them, occupied by granular material. Several of them contain epithelial and granular casts, and there are small vacuoles at the bases of several of the cells. The stroma, as in No. II, is in parts of the medulla very prominent, where the epithelium of the

tubules cut transversely seems to have disappeared, and the canal is either empty or contains red blood corpuscles and debris. The nuclei of the stroma cells stain well, but those of the epithelium vary. In parts, there is a small-celled infiltration of the stroma, but this is infrequent. Many of the tubules on cross section show only one or two cells remaining, and the canal contains shed cells, fibrinous debris, and sometimes red blood corpuscles, revealing transition stages between those with swollen epithelium and granular contents, and those from which the epithelium has disappeared and whose canals are either empty or contain red corpuscles and granular or fibrinous debris.

Flemming sections show that the cells of many of the convoluted tubules and of the ascending loops of Henle contain fat, often in large drops occupying almost the entire cell; in others there are several small droplets, and the nuclei are still visible. The fat in No III is rather less than in No. II, but death was more acute, and very little excretion can have had time to take place.

Pancreas: Vessels congested, stroma somewhat oedematous, necrobiosis slight. Sections stained by haematoxylin and Hansen's modification of van Gieson do not show well-marked nuclei, though the islets of Langerhans are prominent. Giemsa-stained sections do not show sufficient differentiation. Those stained by Flemming and safranin show distinct nuclei, and occasional cells have fat-stained contents. In one part there is a fairly extensive haemorrhage, apparently interstitial in origin, but causing considerable destruction of cells.

Spleen: General congestion of vessels and a few small areas of extravasation are seen; oedema of stroma, which appears to be rather in excess of what is usually present, possibly owing to the swelling and oedema. In the extravasated areas the cells show some necrosis, the nuclei stain poorly, and exhibit some karyolysis; in other parts the cells take the stain well.

Stomach: Macroscopically, congestion, but not marked, though extensive in superficial area. Microscopically, nothing of importance noted.

Lung: Vessels congested; alveoli mostly normal, but here and there are some containing red blood corpuscles, and in other places some fibrinous and granular debris, with shed alveolar epithelium. In the neighbourhood of such are distended alveoli (compensatory emphysema). The bronchioles in parts also show granular matter, with mucus and entangled epithelium and a few leucocytes. No pneumococci or other organisms seen.

Heart-muscle: Minute capillary haemorrhages, here and there, between the muscle fibres. Striation fairly well preserved and nuclei stain well; very little fragmentation.

Flemming-stained sections show black fat granules in some of the fibres; the degeneration, however, does not appear to be extensive, but, unfortunately the solution has not penetrated well.

Thymus: Apparently normal.

Cerebrum: Congestion of vessels of pia, and engorgement of the cerebral capillaries generally, which, here and there, appear to have given way. The pia is oedematous.

Flemming sections reveal a peculiar condition: several of the capillaries show minute droplets stained black, and some of the nerve-cells show a similar condition of the protoplasm. In this animal the capillaries are more affected than the cells (see No. IV).

Cerebellum: Congestion of vessels as in the cerebrum, and in addition, a few of the cells in the sections treated by Flemming's method show black droplets; some of the capillaries also contain these droplets. Nothing else abnormal seen.

In this case we see the symptoms typical of vomiting sickness following rapidly after administration of 'pot-water,' and the post-mortem findings, macroscopical and microscopical, are identical with those of a human case.

In the case of Kitten I, one might, in fact one would, be inclined to infer that death was due to pneumonia, but though, of course, this aspect must be considered, I am not inclined to adopt this view for the following reasons:—

1. The organisms were not very numerous.
2. They were confined to the infected area, which was a small one.
3. The animal had no cough and no apparent respiratory embarrassment before death.
4. The animal died rapidly, with typical vomiting sickness symptoms. It had been to all appearances quite well and running about at noon on March 29th, and died the same evening (about six hours after).
5. There was no enlargement or affection of the bronchial glands.
6. The other organs, liver, kidneys, etc., showed the typical pathological appearances found in cases of death from vomiting sickness.
7. Nos. II and III showed the same changes in the other organs as No. I, but had no pneumococci in spite of considerable congestion of the pulmonary vessels and some of the alveoli, and a definite infarct in the case of No. II.
8. The organisms were not found in the blood-vessels of any other organ, that is, a condition of septicaemia was not present as one would reasonably expect in death arising so acutely, if due to this cause (pneumococcal infection).

The ackee season being practically over, it was becoming a difficult matter to obtain the fruit. However, as we had remaining from the last experiment the extract equivalent to four ackees, we thought it advisable to administer this to another animal, a pup, and if this amount was, as we expected, insufficient to cause death, it would be advantageous to destroy it at an interval of some hours after the last dose and see what may be called an intermediate stage of the disease.

The results were most instructive.

Dog. Female, aged two months; healthy and well nourished; weighing 1850 grammes.

On the 10th May, at 11.40 a.m., 25 c.c. of the extract was administered intragastrically. No symptoms showed themselves till 1 p.m., when the animal was noticed to be unnaturally sleepy; no vomiting had occurred. It had had a good feed of milk a short time previously, and consequently absorption was probably slower. By 2 p.m. the drowsiness was passing off and the animal was obviously recovering. Another 10 c.c. was given. Within two hours the drowsiness had returned, but this was not deep and the puppy could be roused; in fact, it took some food in which about 15 c.c. of the extract was mixed.

The next morning, though not very lively, the animal appeared well. At 11 a.m. 15 c.c. was administered; at 12.20 p.m. it vomited a considerable amount of yellowish watery material, and seemed to be drowsy. No other symptoms occurred between this and 2 p.m., when 25 c.c. was given. After a similar interval ($1\frac{1}{4}$ hours) vomiting again occurred, the animal was somnolent, lying down all the time, drowsy but not comatose. At 4 p.m. the remainder (about 20 c.c.) of the extract was given. In all, as stated, the puppy took the equivalent of three to four ordinary ackees. During the evening it vomited, and, though it took some of the milk provided for it during the night, the animal did not appear well the following morning and was disinclined to move.

I think it was more than probable that it would have died, as it was now refusing all food, but as we had no more extract left, and, as already mentioned, we wished to observe an intermediate stage of the affection, the puppy was rapidly chloroformed at 11 a.m., and the autopsy begun before five minutes past.

Macroscopic appearances post-mortem:—

Liver very pale, almost boxwood in appearance throughout; the *stomach* showed a small congested area towards the cardia, no ulceration; the upper part of the *duodenum* was very slightly congested; the remainder of the intestine was normal. The *mesenteric glands* were enlarged and some of them markedly hyperaemic. *Kidneys* showed a dark surface and the cortex was congested, the pyramids were pale. *Spleen* and *pancreas* showed no obvious change. *Lungs* were generally congested, a few

petechial patches, no infarcts seen. *Heart-muscle* apparently normal. *Brain*, both cerebrum and cerebellum, showed congested vessels on the surface, and possibly also on section.

The *bladder* was full of pale yellow clear urine. No definite albumen reaction was given, but the centrifugalised deposit showed a few hyaline casts and several epithelial and granular ones.

Histological examination:—

Liver: Congestion of vessels, veins and capillaries, both hepatic and portal. The capillaries between the columns of liver cells are full, and, in many cases, they appear to have given way, and there are collections of red blood corpuscles and granular and fibrinous debris between the cell rows. The nuclei throughout have taken the stain moderately well, but nearly all the cells show vacuolation in all stages, from the very minute to a condition in which practically the whole cell is vacuolated. There are in one or two patches small collections of micro-cellular infiltration. Flemming sections show widespread fatty degeneration, the cells containing very many small droplets of fat universally distributed over the lobules, wherever the fluid has penetrated. In a few situations these small droplets have coalesced to form larger masses, but in the majority of cells they are minute, and so numerous that not infrequently the nuclei are obliterated.

Kidney: Vessels congested, and in some situations the engorgement is such that small extravasations have occurred. The glomeruli (as in kitten III) are separated from the capsule by an interval which, in many cases, contain red blood corpuscles, or fibrinous residue. In some of the cells of the glomeruli small vacuoles are seen, but the nuclei on the whole stain well. Some of the tufts appear to have ruptured. The epithelium of the convoluted tubules is swollen and granular, and the canals contain epithelial and granular casts, and occasionally blood. The limits of the cells are often obscured, but on the whole the nuclei are fairly clear; in some cases karyolysis is present. Flemming sections show fatty degeneration of the cells of the convoluted tubules and ascending limbs of the loops of Henle, less of the descending. The fat is situated at the base of the cells. The condition is widespread, but in minute droplets, rarely coalesced to larger ones. In a few instances droplets can also be seen in the glomeruli and cells of Bowman's membrane.

Pancreas: Vessels moderately full of blood; some of the interlobular and interacinar capillaries congested; no extravasations seen. Stroma oedematous; practically no necrobiosis; in one or two very localised situations the cells are indistinct and the nuclei have not stained well, but otherwise, in sections stained by Hansen's method or Giemsa, the gland appears normal. Flemming sections show very widespread, but not severe, fatty degeneration. By this is meant that very many cells (perhaps the majority) show minute fat droplets, but in very few have they become confluent. The cells of the islets of Langerhans are not exempt, but they are less affected than the gland cells proper.

Spleen: Vessels engorged; stroma appears to be in excess of the normal. Nuclei stain well. In various situations extravasated red blood corpuscles are seen, or fibrinous and granular blood residue. Flemming sections show that many of the cells contain minute fat droplets; they are distributed widely throughout the section, but nowhere are they large. This condition has not been noticed in any of the other animals.

Stomach: Nothing much abnormal seen. Small extravasations (petechiae)

at the mucous surface, here and there, and localised hyperaemia. Patchy infiltration of the submucosa, with congestion of capillaries of this layer and the base of the mucosa.

Lung: Small patches of congestion and bronchopneumonia seen; some alveoli contain red blood corpuscles, shed epithelium and a few leucocytes, the neighbouring alveoli being enlarged and distended to compensate. The rest of the organ appears normal, except for capillary congestion. No infarcts present and no micro-organisms seen.

Heart-muscle: No fragmentation; transverse striation well preserved; nuclei stain well. Minute extravasations in parts between the muscle fibres. In the deeper layers of the visceral pericardium, in fact, almost subpericardial, there is a more extensive haemorrhage, which has led to some destruction of the subjacent muscle fibres. This may possibly have been caused when the piece of tissue was taken.

Thymus: Except for some microcellular infiltration of the stroma in a few situations, nothing abnormal seen in the sections. Hassall's corpuscles are not numerous, but, where present, they show up well. The capillaries in parts are congested, but no extravasations are seen.

Mesenteric lymphatic gland: Congestion of vessels and general diffuse hyperaemia. Extravasations of blood scattered in various parts of the section. These are in most instances, but not in all, seen in the interior of the lobules of the gland tissue. The nuclei of the gland cells, except in the area of extravasation stain well. There is some oedema of the stroma.

Cerebrum: The pia appears oedematous, and the vessels are congested. The capillaries of the interior of the section are also full, but no haemorrhages are visible. Flemming sections reveal the curious fact that many of the nerve cells contain minute droplets stained black by the osmic acid. Many of the capillaries also contain fat; in fact, the picture suggests a mixture of degeneration of nerve cells together with (possibly arising from) an embolic condition of the capillaries. I am not at all convinced, however, that these two processes are not distinct, for in parts the capillaries show these droplets while the cells affected may be some distance away, those nearest to the capillary apparently not being affected; on the other hand, in some situations, several cells may show these changes while the adjacent capillaries do not contain the droplets.

Cerebellum: Similar to the cerebrum in congestion of vessels of the meninges and interior, with oedema of the pia. No haemorrhages seen. It may be noted that the cells of Purkinje in some instances show fragmentation of the nucleus, while in a few, the protoplasm is very granular and no nucleus can be made out at all. The majority, however, stain normally. Flemming sections show capillaries containing fat droplets as in the case of the cerebrum, but very few of the cells appear to be affected. Those that are affected are mainly in the close neighbourhood of the capillary from which some emigration of corpuscles had occurred, or whose wall has given way.

It is hoped that further experimental work may be undertaken during the coming winter. This will come within the domain of the chemist rather than that of myself. The aim will be to separate from an aqueous extract of the ackee the toxic principle or principles, and to test the effects of the various isolated constituents on animal subjects.

I am greatly indebted to Mr. Simmons for agreeing to undertake the first part of this work.

VII. ACKEE POISONING

REPORT OF A RECENT CASE OF POISONING BY THE FRUIT
OF THE ACKEE (*Blighia sapida*).

While writing up the last section a most interesting occurrence took place in a country district thirteen miles from Kingston, which not only goes far towards supporting my contention but practically affords the solution of this difficult problem.

On Thursday evening, August 19th, 1915, a family of eight, all of whom were at the time in good health, partook of a meal of ackees taken from a tree which had been 'blown by the hurricane' of the previous week. About two hours later almost every one of the eight complained of feeling sick. Some home remedy (nature not known) was given to all, and they experienced some relief. The following morning they still felt ill. During the day three of them began to vomit, and one, aged 42 years, suffered from convulsions, became unconscious and died the same evening. The others were seen by a medical man, Dr. S. C. Ormsby, who treated them for 'irritant poisoning' and reported the cases as suffering from ackee poisoning. They made good progress.

One member of the family denied that any ackees had been eaten subsequent to the meal on the 19th, but others stated that they had some more on the following day. This would explain the nature of the symptoms in the three individuals mentioned.

The one who died had drunk some of the 'soup.'

During the succeeding six days some of the family still ate ackees for their meals on and off. On Wednesday, 25th, at 6 p.m., M. S., female, 22 years of age, drank the soup and had some of the meal of boiled ackees. About 8 p.m. she vomited, and slightly improved. Later (about 10 p.m.) she had a return of the vomiting, became convulsed, lost consciousness and died shortly after midnight. Another member of the family was also taken ill, but recovered after vomiting.

Having received a telegram relative to this case, I went to the place and carried out a post-mortem examination in the presence of Dr. Ormsby, at noon on the 26th.

The body was that of a well-nourished woman whose age was stated to be 22 years. There was no jaundice or discoloration of conjunctivae; there was no rash, no lice or ticks.

Prior to starting on the dissection, I made a lumbar puncture and obtained a few drops of cerebro-spinal fluid. Two tubes of nasgar were inoculated, and smears were made. The fluid was not present in excess, nor was it under pressure.

Macroscopically:—

Brain: The meninges were congested, and there was some serous effusion over the arachnoid, at the convexity and at the base. Fluid was present in the ventricles, but not in excess. There were no macroscopic signs of disease on section of the brain tissue, except a few petechial points.

Lungs: Congested; no true infarcts, but one part in the left lower lobe appeared to be a little more deeply congested than the surrounding parts.

The lung tissue was frothy, crepitant, and floated in water. The trachea contained a little frothy mucus. Larynx normal. Bronchial glands not enlarged.

The *Myocardium* was possibly a little pale, but the heart appeared otherwise normal; there was no valvular defect. A few small petechiae were seen on the visceral pericardium at the base of the right ventricle.

Thyroid normal in aspect.

Stomach: Showed slight congestion, especially towards the pylorus, where two or three petechiae were visible.

The contents were very small in quantity, and consisted of frothy, grumous material.

The *Duodenum* showed congestion also, but even less marked than that of the stomach.

Nothing abnormal found in the intestines, no worms present.

The *Liver* was of a yellowish red colour, with pale patches, and looked 'fatty.'

The *Spleen* was small but apparently normal, except that the fibrous connective tissue seemed to be in excess. The *Pancreas* was distinctly congested, but there were no visible haemorrhages, and the consistence seemed normal. The *Kidneys:* stellate veins very prominent, capsule transparent, smooth, not adherent. On section of the kidney tissue, pale streaks were seen in the cortex, and the bases of the pyramids were congested. The *Mesenteric glands* were enlarged and some of them hyperaemic.

Microscopically:—

The spinal fluid smears showed no organisms and very few cells, and these were nearly all mononuclear. The culture tubes revealed two colonies of *Staphylococcus albus* in one of them, the other remained sterile. This growth was probably a skin contamination in making the puncture, as the autopsy was performed in the bush, and not under the best bacteriological conditions.

Cultures of the heart-blood remained sterile, and no parasites were seen in the blood smears.

Small pieces of the various organs and tissues were taken (a) in alcohol, (b) in Flemming's solution, and embedded in paraffin. The following pathological changes were seen on examination of the sections:—

Cerebral Cortex: The pia is oedematous and the vessels congested. There is congestion of the vessels of the brain substance, but not in a marked degree. The nerve cells, as a rule, have taken the stain well, and their nuclei are normally situated; occasionally there is displacement of nucleus, with poor staining, and some karyolysis.

Flemming stained sections show that several of the capillaries and larger

vessels contain fat droplets, but these more often appear to be in the cells of the vessel wall than actually within the lumen as emboli.

Many of the nerve cells are seen to contain black droplets (fat). These may be so numerous as to nearly fill the cell, but in the majority the nucleus is still visible; when the number of droplets is large the nucleus may be displaced.

Cerebellum: This shows similar congestion of vessels, but less marked than in the cerebrum. The cells have taken the stain well almost everywhere, but in a few situations the cells of Purkinje show the nucleus eccentric and fragmented; while in one or two no nucleus can be made out at all, and the cells appear necrotic.

Flemming-fixed sections show the same changes as have been described in the cerebrum, but not so extensive nor so marked.

Lung: Marked congestion, vessels all engorged. In parts the alveoli are distended and contain red blood corpuscles, leucocytes, and shed epithelium. The alveolar walls are broken down in places, so that large irregular spaces are produced containing the same elements. The mucous membrane of the bronchioles is swollen and the epithelium is shed into the lumen.

In Giemsa stained sections a few cocci are visible here and there, a post-mortem or accidental contamination.

The above conditions are seen in the congested parts of the lungs; sections from other parts show little except filled capillaries and some shedding of alveolar epithelium.

Flemming treated sections show fat droplets of varied size in various scattered situations. They are mainly present in the walls of the alveoli (pulmonary capillaries), and only rarely have they coalesced to form large drops.

Heart-muscle: The epicardium appears normal; the subepicardial vessels are engorged. Occasionally small microcellular infiltrations of the intermuscular connective tissues are seen, and a similar infiltration beneath the epicardium. The muscle fibres do not show any abnormality, except a few in which minute vacuoles are visible. The transverse striation is well preserved, and there is little or no fragmentation of the fibres. The nuclei stain well on the whole, but here and there are some which show karyolysis and have stained badly.

Flemming sections show that several of the fibres contain fat droplets; some only a few, others are nearly filled with them.

Liver: No alteration seen in the capsule. The vessels in parts exhibit a certain degree of congestion, but no haemorrhages are seen. The periportal connective tissue shows some apparent increase, but this may be due to contrast with the vacuolated liver cells.

The liver tissue itself is almost unrecognizable owing to the intense and widespread fatty metamorphosis. Some of the cells of the parenchyma (the minority) show a number of small vacuoles, but nearly all are distended by a large fat drop, the cell nucleus being thrust to the periphery. The nuclei of some of the cells take the stain well, others are barely visible, while others, again, show karyolysis. The fatty change is intense, and distributed over the whole section almost equally; in some cases, however, the fat drops in the neighbourhood of the portal vessels are larger than those in the interior of the lobule, but this is by no means always the case. Few recognizable liver cells are left. This intense change may be explained by the fact that the patient had been taking ackees, on and off, during the week, and had been ill after the meal a week previously, and had taken the 'soup' (extracted poison?) again the same evening before she died.

Flemming sections do not reveal anything further, except to prove that the vacuolization is due to fatty metamorphosis.

Kidney: General congestion, and in one part of the section actual extravasation has occurred. The glomeruli are swollen, and in a few, between the tuft and Bowman's capsule, there is granular (corpuscular?) debris. The cells of many of the glomeruli are necrobiotic, and into the glomerular spaces, in some instances, the lining cells of Bowman's membrane are shed. The intertubular connective tissue is slightly increased. There is an intense parenchymatous nephritis; many of the convoluted tubules show disorganisation of structure—the cell-limits are undefined, the nuclei are hardly recognisable (in parts they are not visible at all), the cells are granular, vacuolated, and necrobiotic. Many of the tubules contain red blood corpuscles or their residue, and in some the secreting epithelium has quite disappeared. In others, the lumen is occupied by blood, or by granular degenerated epithelium. The lining epithelium of many of the convoluted tubules and of the ascending limbs of the loops of Henle show extensive vacuolization, especially marked at the bases of the cells. The descending limbs of Henle's loops have not escaped, but they are less affected.

Flemming sections show extensive fatty metamorphosis. This has barely affected the Malpighian tufts at all, a few black dots only are seen in them, and in the cells of Bowman's capsule occasionally. But in the epithelium of the convoluted tubules and the ascending limbs of the loops of Henle, the change is very marked; less, but still extensive, in the descending limbs.

Spleen: The capsule is increased somewhat in thickness, and the interstitial tissue throughout the section is in excess of that normally present. There is general congestion, but no haemorrhages are seen; here and there, however, are patches of what appear to be fibrinous and possibly corpuscular debris scattered amongst the parenchyma cells.

Flemming sections reveal minute droplets of fat scattered in various parts of the section. They are present in the cells of the parenchyma, but to a considerably less extent than in the fibrous trabeculae, though nowhere are they numerous.

Pancreas: Vessels congested, but no extensive haemorrhages seen. There are one or two spots where small extravasations have occurred. The interlobular connective tissue appears increased and possibly somewhat oedematous. There is a similar condition of the interacinar connective tissue.

The glandular epithelium shows very extensive necrobiosis. The cell-limits are badly defined, the nuclei in many cannot be seen, in others there is karyolysis. In fact, in a few situations only is the normal pancreatic epithelium visible. Many of the cells appear to contain minute vacuoles. The islets of Langerhans are also affected, and can rarely be distinguished in the tissue. Flemming treated sections reveal the fact that barely any normal pancreatic tissue is left, the fatty metamorphosis and necrobiosis are so marked. The nuclei are visible, but otherwise the cells appear to be filled by black-stained fat droplets, which often have coalesced so as to practically obliterate the cell.

Lymph-gland (mesenteric): There is general congestion of the gland; the vessels are engorged, and in one or two situations the capillaries appear to have given way. The stroma is oedematous. The majority of the cells stain well, but in many parts are areas where the cells are necrosed—they are granular and have barely stained at all. Such occur in considerable patches, but also, in other parts, are intermixed with cells which have stained normally.

In this case, then, we have a definite history of a patient in good health partaking of a meal of ackees from a bruised limb. She, with other members of the family, suffered from vomiting, and

recovered. A week later another meal was prepared with fruit from the same tree. The patient drank the 'soup' and also ate some of the solid. Two hours later the symptoms made their appearance and ran their course to a fatal termination in six hours or so, and at the post-mortem the changes which have been described above and in the last section were revealed. In this case the term 'Vomiting Sickness' was not used from first to last, but the case showed typically the onset, course, and pathological changes of that disease.

Before finally summing up the points treated in detail in this paper, a brief consideration of certain peculiarities and characteristics of the affection may be given. This will demonstrate the similarity—a similarity which, in my opinion, amounts to identity—between vomiting sickness on the one hand and the experimental results in animals of administration of ackee extract and the clinical case of definite ackee poisoning on the other:—

- (1) Peculiar seasonal prevalence.
- (2) Its confinement to Jamaica so far as is known.
- (3) Sudden onset of symptoms in apparent good health, and in the well-nourished as in the emaciated.
- (4) The rapid and complete recovery of non-fatal cases.
- (5) Affection of several members in one house or close neighbours in a settlement.
- (6) Its vastly greater preponderance in children.
- (7) No preference as regards sex.
- (8) White children practically never attacked, East Indians rarely.
- (9) The pathological changes set up.

(1) *Peculiar Seasonal Prevalence.* The disease corresponds exactly with the main ackee season, when other fruits and natural foods are relatively scarce. This year the ackee season has continued longer than in previous years in my experience; instead of ending in March or early in April the fruit has been abundant till well on in May; and, owing to an exceptionally good season of rains at unusual times, some trees are even bearing now (August). Vomiting sickness cases have been reported this year in greater numbers than last, and over a more prolonged period. This correspondence between the ackee season and vomiting sickness cases is borne out by my records for three or four years past.

(2) *Limitation to Jamaica.* As Seidelin states (p. 455 of his report): 'The Government of Jamaica made enquiries in a circular letter to the authorities of other West Indian Islands, with regard to the possible occurrence of vomiting sickness in other parts of the West Indies. Only two of the answers are quoted by Ker in the Annual Report (1906), the others being in the negative. The one positive answer is from Finlay, who mentions an outbreak of twenty-five similar cases with five deaths amongst soldiers in Cuba; he suggests that the disease is cerebro-spinal meningitis. No particulars are given in the quotation. The other positive answer deals with a disease (in Haiti) which has obviously no essential resemblance to vomiting sickness.'

I have made enquiries from those who are in a position to know, and I am told that ackees do not grow, at all events to any extent, in any other island, and the limitation of the disease to Jamaica would thus find explanation. One or two trees only are growing in other islands, but they are looked upon as curiosities and are not used for food.

It is true that some ackees are shipped to Colon from Jamaica, but only such as are ripe and open or which open within twenty-four hours of leaving Jamaica.

(3) *Sudden onset of symptoms in apparent good health* and in well-nourished, not necessarily emaciated, subjects. The symptoms, as has been pointed out more than once in the foregoing pages, are those of an acute intoxication, and those symptoms would depend not so much on the general well-being of the subject as on the dose of the poison and the condition of the stomach (whether empty or full) and its readiness for absorption.

(4) *The rapid and complete recovery of non-fatal cases.* This is practically answered in the last paragraph; an acute vegetable poison is taken in small quantity, is got rid of by vomiting, and the patient completely recovers. This, one sees, for example, in mushroom poisoning.

(5) *Affection of several members practically simultaneously in one house*, or close neighbours in a settlement. These facts are both well supported in the Montego Bay report. Several members are affected in one house because they partake of a similar meal, or the same articles of diet are cooked up together and shared in common.

The graded acuteness of symptoms mentioned in cases where the adults ate the solid, the older children the semi-solid and absorbed watery extract, the younger ones the soup or pot-water, further bears this out.

Close neighbours in a settlement are affected because the trees are in and about the settlement and all share in the produce.

(6) *The vastly greater preponderance in children.* This has been shown in my previous reports, and spoken of in Section III. This is explained by the fact that they are given the pot-water, the most toxic part—in short, an extracted poison—and that the lethal dose of a poison is far smaller in a child, generally speaking, than in the case of an adult; and, lastly, adults know the risks of eating unopened ackees, while children naturally do not.

(7) *No preference as regards sex.* One would not expect a vegetable poison to exercise any sex-selective powers.

(8) *Attacking the West Indian native in much greater numbers than East Indian or White.* The coolie diet consists mainly of rice and split peas, often in the form of curry. They are also fond of green fruit, such as green mangoes, guavas, jack-fruit. They never eat pork or beef, but like goat when they can get it. After they have been in Jamaica for some time, some of them take gradually to eating 'salt-fish, but never to the same extent as the West Indian native.

They very rarely indeed eat ackee; a few may do so after they have served their time and are out of their indentures, and have settled in the island, but even then it is not a common article of diet with them.

One who has had a good deal to do with the East Indian in Jamaica tells me that though he believes, as just stated, that the older coolies occasionally use ackees, he himself has never yet seen them eat it.

The White buys his ackees in the market, where he can see and select his purchases; or, greater safeguard still, many of them will only eat ackees which are gathered carefully off their own trees.

(9) *The Pathological Changes set up.* These have been already dealt with, and need not be repeated. Some of the specimens from previous cases of vomiting sickness, those from the animals treated with ackee extract, and those from the case of ackee poisoning

described above, could not be distinguished from each other under the microscope, the changes set up are similar. Variations between cases are explicable by the poison acting more particularly on one organ. Thus, the congestion and even haemorrhages which occur may affect particularly the pancreas, for instance, and from this point of view two cases of vomiting sickness reported to me this year in which acute haemorrhagic pancreatitis was found might possibly have after all been cases of true vomiting sickness, the poison acting mainly on this organ. In others the liver may show marked changes due to absorption of the poison in larger doses from an empty stomach, and so on. There is no need to labour this point.

VIII. SUMMARY

1. The term 'Vomiting Sickness' has been used for many years as a comprehensive name for various diseases, including cerebro-spinal meningitis, gastritis, gastro-enteritis, worms, malaria; in fact, practically any disease occurring in the cooler months and associated with vomiting and convulsions.

2. During the last ten years opinions have been expressed to the effect that there is an affection called vomiting sickness whose course of symptoms and post-mortem changes are not those of any known disease.

3. The death rate from this affection is exceedingly high, 80 per cent. to 90 per cent., and a fatal termination occurs in a few hours.

4. The first systematic investigation into the affection was undertaken in 1912 by Captain (now Major) T. J. Potter, R.A.M.C., who came to the conclusion 'that the majority of deaths ascribed to the so-called vomiting sickness are due to yellow fever.'

5. To this succeeded the 'meningitis era,' a recrudescence of the older idea that some cases at all events included under the term 'vomiting sickness' died from cerebro-spinal meningitis.

6. Seidelin's investigation took place the following year (1913), but, though he was the first to give a detailed description of the morbid anatomy, he did not succeed in solving the question of causation. He showed, however, that there was a definite unexplained condition comprising the majority of cases reported as vomiting

sickness, and that the condition was neither yellow fever nor cerebro-spinal meningitis.

7. Investigations into a typical and severe outbreak at Montego Bay in February, 1915, revealed the fact that in a majority of the cases in which a reliable history was obtainable, ackees formed part of the last meal taken in health, and that this article of food could not be excluded in a single case.

8. Persons taking the 'soup' or 'pot-water' made with ackees in certain conditions showed the most acute symptoms; the onset occurred in two hours, and death nearly always resulted.

9. 'Salt-fish,' a frequent article of diet, is in the country parts used as a euphemism for 'salt and ackee.'

10. Ackees under certain conditions are undoubtedly poisonous; among such conditions are: (i) Unopened ackees; (ii) ackees picked from a decayed, bruised, or broken branch; (iii) ackees which have not opened naturally, but which have been forced open; (iv) ackees with a soft spot in an otherwise apparently sound fruit.

11. Much of the poison is extracted by boiling with water.

12. The symptoms of a case of typical vomiting sickness are: Initial vomiting (gastric in origin) coming on in apparently perfect health; a period of a few hours' improvement, succeeded by secondary vomiting (cerebral) rapidly followed by convulsions, coma, and death. The average total duration of illness is twelve and a half hours.

13. Initial, or secondary, vomiting, or convulsions may be absent, but not in a large percentage.

14. Recovery, so far as I am aware, has never occurred when once convulsions set in or coma; and, as a corollary to this, in no cases which recover are convulsions seen.

15. The affection is largely one of childhood, and shows no predilection for sex.

16. A reasonable interpretation of the symptoms is: Some poison is taken, or some substance which acts as a poison after it enters the stomach. If the initial vomiting is sufficient to get rid of this substance no further symptoms occur and recovery rapidly ensues. If this is not the case, there is an interval—a more or less quiescent period of absorption—after which there follow symptoms due to the action of the poison on the higher centres—secondary (cerebral) vomiting, convulsions, drowsiness, coma, and death.

17. In rare instances the cerebral symptoms are those first noticed (convulsions, drowsiness, coma); there is no preceding vomiting—the so-called 'vomiting sickness without vomiting.'

18. The pathological changes consist chiefly of general hyperaemia and a tendency to haemorrhages in various organs, fatty metamorphosis especially in liver and kidneys, and necrobiotic changes of epithelia in liver, kidneys, and pancreas.

19. Micro-organisms are rarely found in true vomiting sickness cases, and, when present, are probably accidental and have no pathological significance.

20. Intragastric administration of an extract, made by boiling unopened ackees with water, produced in three kittens and one pup the symptoms and pathological changes seen in cases of vomiting sickness.

21. A case of ackee poisoning in a human subject exhibited the same symptoms, course, and post-mortem changes, macroscopical and microscopical, as (a) human vomiting sickness cases, and (b) animals to whom an aqueous extract of unopened ackees had been administered.

22. The characteristics of 'vomiting sickness,' viz. :—

- (i) Peculiar seasonal prevalence;
- (ii) Its confinement to Jamaica, so far as is known;
- (iii) The sudden onset of symptoms in apparent good health, and in the well-nourished as in the emaciated;
- (iv) The rapid and complete recovery of non-fatal cases;
- (v) The affection of several members in one house, or close neighbours in a settlement;
- (vi) The vastly greater preponderance in children;
- (vii) The absence of preference as regards sex;
- (viii) The rarity of occurrence in White children and East Indians;
- (ix) The pathological changes induced;

all find explanation in the view that the condition is an acute intoxication by unopened or unwholesome ackees—the fruit of *Blighia sapida*.

In conclusion, I desire to express my acknowledgments to the Hon. Dr. J. Errington Ker, Superintending Medical Officer, for allowing me to have access to the records of the department of which

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TABLE I.—Vomiting Sickness.
All cases reported of which the histories were reliable.

	Under 1 year	1-2 years	2-3 years	3-4 years	4-5 years	5-6 years	6-7 years	7-8 years	8-9 years	9-10 years	10-11 years	11-12 years	12-13 years	13-14 years	14-15 years	15-16 years	16-17 years	17-18 years	18-19 years	19-20 years	Over 20 years	Total
Fatal Cases	2	5	15	11	10	10	12	6	7	1	2	3	4	1	—	—	—	1	—	—	1	91
Male
Female	4	8	11	22	18	5	10	5	6	6	4	2	2	2	1	1	—	1	1	1	16	126
Total	6	13	26	33	28	15	22	11	13	7	6	5	6	3	1	1	—	2	1	1	17	217
Recoveries	—	1	1	2	—	2	1	—	3	2	—	—	1	—	1	—	—	—	—	—	—	14
Male
Female	1	2	2	3	4	1	1	—	2	—	2	2	1	1	—	—	—	—	—	—	12	34
Total	1	3	3	5	4	3	2	—	5	2	2	2	2	1	1	—	—	—	—	—	12	48
Grand Total	7	16	29	38	32	18	24	11	18	9	8	7	8	4	2	1	—	2	1	1	29	265

TABLE II.—Meningitis cases*
Amongst those reported as Vomiting Sickness.

	Under 1 year	1-2 years	2-3 years	3-4 years	4-5 years	5-6 years	6-7 years	7-8 years	8-9 years	9-10 years	10-11 years	11-12 years	12-13 years	13-14 years	14-15 years	15-16 years	16-17 years	17-18 years	18-19 years	19-20 years	Over 20 years	Total
Fatal Cases	—	1	2	3	2	3	1	4	2	—	1	—	2	1	—	—	—	1	—	—	—	23
Male
Female	4	1	2	4	3	1	2	—	1	2	—	—	—	—	—	1	—	—	—	1	1	23
Total	4	2	4	7	5	4	3	4	3	2	1	—	2	1	—	1	—	1	—	1	1	46
Recoveries	—	—	1	—	—	—	1	—	1	—	—	—	—	—	1	—	—	—	—	—	—	4
Male
Female	1	—	1	—	—	—	—	—	1	—	—	2	—	—	—	—	—	—	—	—	1	6
Total	1	—	2	—	—	—	1	—	2	—	—	2	—	—	1	—	—	—	—	—	1	10
Grand Total	5	2	6	7	5	4	4	4	5	2	1	2	2	1	1	1	—	1	—	1	2	56

* That is, cases exhibiting symptoms of Meningitis, and in whose spinal fluid a Gram-negative diplococcus was found.

TABLE III.—Vomiting Sickness less 'Meningitic' * cases.

	Under 1 year	1-2 years	2-3 years	3-4 years	4-5 years	5-6 years	6-7 years	7-8 years	8-9 years	9-10 years	10-11 years	11-12 years	12-13 years	13-14 years	14-15 years	15-16 years	16-17 years	17-18 years	18-19 years	19-20 years	Over 20 years	Total
Male	2	4	13	8	8	7	11	2	5	1	1	3	2	—	—	—	—	—	—	—	1	68
Female	—	7	9	18	15	4	8	5	5	4	4	2	2	2	1	—	—	1	1	—	15	103
Total	2	11	22	26	23	11	19	7	10	5	5	5	4	2	1	—	—	1	1	—	16	171
Male	—	1	—	2	—	2	—	—	2	2	—	—	1	—	—	—	—	—	—	—	—	10
Female	—	2	1	3	4	1	1	—	1	—	2	—	1	1	—	—	—	—	—	—	11	28
Total	—	3	1	5	4	3	1	—	3	2	2	—	2	1	—	—	—	—	—	—	11	38
Grand Total	2	14	23	31	27	14	20	7	13	7	7	5	6	3	1	—	—	1	1	—	27	209

* For details as to age, etc., see Table II.

TABLE IV.—Vomiting Sickness,* after deduction of Meningitis and 'probable Meningitis' cases.

	Under 1 year	1-2 years	2-3 years	3-4 years	4-5 years	5-6 years	6-7 years	7-8 years	8-9 years	9-10 years	10-11 years	11-12 years	12-13 years	13-14 years	14-15 years	15-16 years	16-17 years	17-18 years	18-19 years	19-20 years	Over 20 years	Total
Male	2	4	13	6	7	7	11	2	4	1	1	2	2	—	—	—	—	—	—	—	1	63
Female	—	7	7	15	13	4	8	4	5	4	3	2	2	2	1	—	—	1	1	—	15	94
Total	2	11	20	21	20	11	19	6	9	5	4	4	4	2	1	—	—	1	1	—	16	157
Male	—	1	—	2	—	2	—	—	2	1	—	—	1	—	—	—	—	—	—	—	—	9
Female	—	2	1	3	4	1	1	—	1	—	2	—	1	1	—	—	—	—	—	—	11	28
Total	—	3	1	5	4	3	1	—	3	1	2	—	2	1	—	—	—	—	—	—	11	37
Grand Total	2	14	21	26	24	14	20	6	12	6	6	4	6	3	1	—	—	1	1	—	27	194

* Six of these were probable only (see text).

TABLE V.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	DIAGNOSIS			Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated						Vomiting Sickness	V.S.	Meningitis			
1	B.A.M.	2	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	5 hours	Slight twitchings, no general convulsions
2	C.R.	3	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	15 "	
3	L.H.	18	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	20 "	
4	M.H.	8	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	36 "	
5	M.J.	1 $\frac{1}{2}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	2 "	
6	L.M.	4	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	3 "	
7	—R.	3 $\frac{1}{2}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	7 "	
8	E.C.	6	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	R.	7 "	Vomiting only, others in family typical
9	B.C.	1 $\frac{3}{4}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	5 hours	
10	C.N.	4	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	4 "	
11	L.W.	2 $\frac{1}{2}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	15 "	No mention of convulsions
12	P.H.	2 $\frac{1}{2}$	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	12 hours	Duration not stated, comatose when seen
13	L.McE.	25	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	10 "	Appeared to have abdominal pain, but not certain
14	L.H.	25	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	12 "	'Slight fever,' temperature not stated
15	M.W.	1 $\frac{1}{2}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	12 "	No mention of vomiting; p.m.
16	S.M.S.	3 $\frac{1}{2}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	5 "	No mention of vomiting; p.m.
17	C.T.	8	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	8 "	No definite convulsions
18	A.G.	3 $\frac{1}{2}$	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	44 "	Prolonged quiescent interval, 33 hours
19	A.L.	6	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	8 "	
20	J.E.B.	3 $\frac{1}{2}$	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	10 "	Abdominal discomfort, no real pain
21	D.G.	3	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	3 "	No secondary vomiting
22	W.S.	7	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	6 "	
23	F.P.	8	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	12 "	
24	M.H.	17	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	10 "	
25	L.M.	5	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	26 "	Lapsed into coma after initial vomiting, no convulsions noticed
26	M.A.P.	25	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	24 "	Twitching only, no general convulsions
27	L.T.	8	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	4 "	Convulsions and coma only mentioned; rigidity of neck only during convulsions
28	S.R.	6 $\frac{1}{2}$	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	2 "	Coma soon after vomiting; (no 'initial' vomiting)
29	L.E.	6	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	R.	2 hours	Child of this one died. Mother vomiting and recovered
30	H.C.	37	F.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	20 "	
31	P.W.	6	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	20 "	
32	D.C.	11	M.	—	—	—	—	—	++	—	—	++	—	+	—	—	D.	20 "	

TABLE V.—continued.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	DIAGNOSIS				Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated						Vomiting Sickness	? V.S.	Meningitis	? M			
33	C.L.	1	F.	+	+	+	+	+	+	+	+	+	+	+	D.	1 hour	History uncertain, became so acute
34	L.R.	2	M.	+	+	+	+	+	+	+	+	+	+	+	D.	2 hours	
35	O.F.	25	F.	+	+	+	+	+	+	+	+	+	+	+	D.	12 "	
36	—F.	4	F.	+	+	+	+	+	+	+	+	+	+	+	D.	4 "	
37	M.	3	F.	+	+	+	+	+	+	+	+	+	+	+	D.	6½ "	
38	L.W.	21	F.	+	+	+	+	+	+	+	+	+	+	+	D.	2 "	'Vomiting Sickness without vomiting'
39	S.N.	2½	M.	?	...	?	+	...	+	+	+	+	D.	? 38 "	History incomplete
40	K.L.W.	2	F.	+	...	+	+	...	+	+	+	+	D.	3 "	'Vomiting Sickness without vomiting'
41	J.W.	2½	M.	+	...	+	+	...	+	+	+	+	D.	3 "	Diplococcus present
42	A.L.	7	M.	+	...	+	+	...	+	+	+	+	D.	21 "	Diplococcus present
43	C.D.	1	F.	+	...	+	+	...	+	+	+	+	D.	? 1 week	
44	H.B.	3	M.	+	...	+	+	...	+	+	+	+	D.	? 2 weeks	Diplococcus present
45	S.C.	17	M.	+	...	+	+	...	+	+	+	+	D.	? "	Diplococcus present; ill on and off for a month
46	R.P.	3½	F.	+	...	+	+	...	+	+	+	+	D.	9 hours	Diplococcus present
47	C.P.	8	M.	+	...	+	+	...	+	+	+	+	D.	10 "	All of one family, the
48	E.P.	15½	F.	+	...	+	+	...	+	+	+	+	D.	12 "	'Peart' series, see text
49	V.P.	11	F.	+	...	+	+	...	+	+	+	+	R.	...	Diplococcus present
50	O.S.	6	M.	+	...	+	+	...	+	+	+	+	R.	...	Diplococcus present; ill for several days
51	P.McM.	4	F.	+	...	+	+	...	+	+	+	+	D.	8 hours	Diplococcus present
52	A.P.	2	F.	+	...	+	+	...	+	+	+	+	R.	...	One family; attended hospital afterwards for some time; others died at home
53	E.P.	1½	F.	+	...	+	+	...	+	+	+	+	R.	...	Vomiting and headache only, but reported as Vomiting Sickness
54	V.P.	8	F.	+	...	+	+	...	+	+	+	+	R.	...	History indefinite, very acute
55	H.P.	25	F.	+	...	+	+	...	+	+	+	+	R.	...	
56	J.L.B.	4	M.	+	...	+	+	...	+	+	+	+	D.	2 hours	
57	L.H.G.	1½	M.	+	...	+	+	...	+	+	+	+	D.	1 hour	
58	M.B.	2½	F.	+	...	+	+	...	+	+	+	+	D.	8½ hours	

TABLE V.—continued.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	DIAGNOSIS			Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated						Vomiting Sickness	? V.S.	Meningitis	? M		
59	E.H.	19	F.	+	-	-	-	+	+	..	+	+	+	+	-	15 days	Diplococcus present. not stated
60	C.M.	13	M.	Through out	-	..	+	?	..	+	..	+	+	+	-	11 hours	Diplococcus present
61	C.M.A.	4	F.	Through out	Through out	+	+	+	..	+	+	+	-	15 "	Diplococcus present
62	H.W.	6	F.	+	+	+	+	..	+	+	+	-	4 "	Diplococcus present
63	A.J.	2	M.	-	-	+	+	+	+	..	+	+	+	-	6 "	No convulsions
64	J.H.	3	M.	-	+	+	+	+	+	..	+	+	+	-	15 "	History incomplete; comatose when seen
65	O.D.	6	F.	+	?	+	+	+	..	+	+	+	-	? 24 "	
66	S.A.H.	3	F.	-	+	+	At intervals throughout	+	+	+	..	+	+	+	-	? 24 "	Diplococcus present
67	R.T.	6	F.	?	..	+	At intervals throughout	+	+	+	..	+	+	+	-	18 1/2 "	
68	D.D.	11	M.	+	-	+	At intervals throughout	+	+	+	..	+	+	+	+	48 "	
69	L.P.	5	M.	+	+	+	+	+	..	+	+	+	-	9 1/2 "	No vomiting
70	A.S.	2	F.	-	-	+	+	+	..	+	+	+	-	3 "	Diplococcus present
71	R.C.C.	8	F.	-	-	-	-	+	+	+	..	+	+	+	-	15 "	
72	G.McL.	4	F.	-	-	+	+	+	+	+	..	+	+	+	-	6 hours	No secondary vomiting recorded
73	J.P.	4	F.	..	+	+	-	+	+	+	..	+	+	+	-	13 "	Very acute, vomiting only recorded
74	J.A.D.	9	F.	+	+	+	+	..	+	+	+	-	1 1/2 "	
75	J.H.	1 1/2	F.	+	+	+	+	..	+	+	+	-	12 "	
76	A.C.	5 1/2	F.	+	+	+	+	100°	+	+	+	-	12 "	
77	M.A.	4	F.	+	+	+	+	..	+	+	+	-	10 "	
78	A.P.D.	8	F.	+	+	+	..	+	+	+	-	8 "	Diplococcus present
79	G.M.F.	5	F.	Through out	Through out	+	+	+	..	+	+	+	-	4 "	Meningitis p.m.
80	O.B.	3	M.	Through out	Through out	+	+	+	..	+	+	+	-	12 "	Diplococcus present
81	Z.E.J.	7	M.	Through out	Through out	+	+	+	..	+	+	+	-	3 days	Diplococcus present
82	C.R.	5	F.	+	+	+	+	+	..	+	+	+	-	14 hours	Diplococcus present
83	M.R.	7	F.	+	+	+	+	+	..	+	+	+	-	7 "	
84	I.V.	5	M.	+	+	+	+	+	..	+	+	+	-	3 "	Diplococcus present, see text
85	B.D.	4	F.	Through out	Through out	+	+	+	..	+	+	+	-	3 1/2 "	Diplococcus present. Vomiting too acute to notice other symptoms
86	V.C.	2	F.	+	+	+	+	+	..	+	+	+	-	11 "	
87	E.S.	6	F.	+	+	+	+	+	..	+	+	+	-	3 "	
88	L.F.	4	F.	+	+	+	+	+	..	+	+	+	-	23 "	
89	A.J.	6	M.	+	+	+	+	+	..	+	+	+	-	3 "	
90	J.J.	4	M.	+	+	+	+	+	..	+	+	+	-	25 "	One family; two younger died, two older recovered
91	M.J.	10	F.	+	+	+	+	+	..	+	+	+	-	..	
92	G.J.	8	M.	+	+	+	+	+	Slight	+	+	+	-	..	

TABLE V.—continued.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	Vomiting Sickness	DIAGNOSIS		Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated							V.S.	Meningitis			
93	J.H.	3	F.	Through out	+	++	...	++	++	...	+	+	+	D.	10 hours	Diplococcus present
94	A.H.	4	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	9 1/2 "	Diplococcus present
95	S.B.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	1 1/2 "	Diplococcus present
96	J.F.	5	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	12 "	Diplococcus present
97	R.F.	9	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	36 "	Diplococcus present
98	V.L.C.	4	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	24 "	'Typical C.S. Meningitis p.m.,' see text
99	V.F.	2	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	17 "	
100	J.W.	12	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	8 "	
101	D.W.	10	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	25 "	
102	A.R.	24	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	8 "	
103	C.R.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	18 "	
104	L.J.R.	3 1/2	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	14 "	
105	J.G.	8	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	10 "	
106	H.S.	3 1/2	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	...	Diplococcus present
107	A.S.	3 1/2	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	...	Diplococcus present; no history sent
108	S.S.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	R.	...	
109	A.W.	1 1/2	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	R.	...	
110	F.B.	1 1/2	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	14 hours	Diplococcus present, see text
111	E.M.	3 1/2	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	12 "	Diplococcus present
112	J.B.	5	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	?	Vomiting only symptom mentioned
113	E.S.	7	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	23 hours	History of M., but no specimens sent
114	M.C.	3 1/2	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	?	Diplococcus present
115	L.F.	4 1/2	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	6 "	
116	B.W.	12	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	3 days	
117	M.H.	27	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	2 1/2 "	
118	J.J.	5	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	2 "	Recurrent vomiting only mentioned
119	D.C.	12	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	?	Diplococcus present
120	E.W.	35	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	24 hours	No convulsions seen
121	E.W.	60	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	?	No convulsions seen
122	T.C.	24	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	14 hours	
123	J.H.	6	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	12 "	No convulsions seen
124	F.P.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	12 "	Diplococcus present, see text
125	J.B.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	4 "	Vomiting only mentioned in history
126	K.C.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	9 "	
127	J.E.S.	14 1/2	M.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	17 "	
128	A.B.	3	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	1 "	
129	Z.P.	4	F.	Through out	Through out	...	++	...	++	++	...	+	+	+	D.	24 "	Diplococcus present

TABLE V.—continued.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	DIAGNOSIS				Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated						Vomiting Sickness	V.S.	Meningitis	M			
130	G.A.M.	9	F.	+	+	...	+	+	...	+	D.	11 hours	Diplococcus present Diplococcus present } Brothers
131	D.T.	3½	F.	+	+	...	+	+	...	+	R.	"	
132	W.T.	5	F.	+	+	...	+	+	...	+	R.	"	
133	G.A.	4½	F.	+	+	...	+	+	...	+	R.	"	
134	J.A.	9	M.	+	+	+	...	+	R.	"	Diplococcus present Diplococcus present } Brothers
135	A.S.	1½	M.	+	+	+	...	+	R.	"	
136	J.S.	3	F.	+	+	+	...	+	R.	"	
137	A.T.	14	M.	+	At intervals	...	+	+	...	+	R.	"	
138	V.T.	8	M.	+	At intervals	...	+	+	...	+	R.	"	Diplococcus present Diplococcus present } Brothers
139	R.H.	8	M.	+	+	+	...	+	R.	"	
140	L.M.	10	F.	+	+	+	...	+	R.	"	
141	R.E.	7	M.	+	Througout	...	+	+	...	+	R.	"	
142	M.F.	Adult	F.	+	Througout	...	+	+	...	+	D.	"	Diplococcus present Diplococcus present } Brothers
143	R.T.	11	F.	+	At intervals	...	+	+	...	+	D.	"	
144	M.S.	Adult	F.	+	+	+	...	+	R.	"	
145	J.McL.	2½	F.	+	+	+	...	+	R.	"	
146	L.M.	12	F.	+	+	+	...	+	R.	"	Diplococcus present.
147	W.P.	5½	M.	+	+	+	...	+	R.	"	
148	A.M.	5	F.	+	At intervals	...	+	+	...	+	R.	"	
149	D.W.	3½	M.	+	Througout	...	+	+	...	+	D.	"	
150	J.W.	4	F.	+	+	+	...	+	D.	8 hours	No mention of convulsions
151	G.F.	5	F.	+	+	+	...	+	D.	"	
152	C.B.	3	M.	+	+	+	...	+	D.	5 "	
153	C.N.	5½	M.	+	+	+	...	+	D.	"	
154	A.N.	12	M.	+	+	+	...	+	R.	"	Diplococcus present Diplococcus present } Brothers
155	C.N.	9	M.	+	+	+	...	+	D.	20 hours	
156	E.B.	6	M.	+	+	+	...	+	D.	18 "	
157	L.A.M.	3	M.	+	+	+	...	+	R.	"	
158	G.B.	1½	F.	+	+	+	...	+	D.	37 hours	Diplococcus present Diplococcus present } Brothers
159	E.B.	2	M.	+	+	+	...	+	D.	"	
160	H.M.	1½	M.	+	At intervals	...	+	+	...	+	D.	"	
161	A.F.	25	F.	+	+	+	...	+	R.	50 hours	
162	C.C.	20	M.	+	+	+	...	+	D.	"	No mention of convulsions
163	P.B.	11	M.	+	+	+	...	+	D.	"	
164	E.M.	11	F.	+	+	+	...	+	D.	"	
165	H.P.	2	M.	+	+	+	...	+	D.	"	
166	D.D.	10	M.	+	At intervals throughout	...	+	+	...	+	D.	14½ "	? Vomiting Sickness, without vomiting, Diplococcus present

TABLE V.—continued.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	Vomiting Sickness	DIAGNOSIS		Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated							V.S.	Meningitis			
167	D.P.	4	M.	—	+	At intervals throughout	+	...	+	...	+	+	...	+	—	+	D.	14 hours	Diplococcus present
168	N.M.	8	M.	+	+	+	—	+	D.	33 "	Diplococcus present
169	C.G.	5	M.	+	—	+	D.	8 "	No secondary vomiting
170	C.B.	4	M.	At intervals throughout	+	...	+	...	+	+	...	+	—	...	D.	14 "	Probably M., no specimens sent
171	I.S.	2	F.	—	—	...	+	...	+	+	+	+	—	...	D.	12 "	Probably M., no specimens sent
172	I.B.	40	F.	+	+	...	+	+	—	...	D.	27 "	Probably M., no specimens sent
173	E.M.Y.	2½	F.	+	+	...	+	+	—	...	D.	5 "	Probably M., no specimens sent
174	P.B.	4	F.	At intervals throughout	+	...	+	+	—	...	D.	16 "	Probably M., no specimens sent
175	E.McD.	6	M.	+	+	...	+	+	—	...	D.	3½ "	Lapsed into coma without convulsions
176	G.McD.	8	F.	+	+	...	+	+	—	...	D.	40 "	
177	L.L.	12	F.	+	+	...	+	+	—	...	D.	3 days	
178	P.H.	13	F.	+	+	...	+	+	—	...	R.	...	
179	M.B.	3	M.	Through out	+	...	+	+	—	...	D.	?	
180	C.W.	2½	F.	At intervals throughout	+	...	+	+	—	...	D.	4½ hours	Diplococcus present, atypical Diplococcus present
181	C.B.	2½	F.	?	+	...	+	+	—	...	D.	?	
182	G.T.	5	M.	+	+	...	+	+	—	...	D.	13 hours	Reported as Vomiting Sickness, but history uncertain
183	E.P.	2	M.	Through out	+	...	+	+	—	...	D.	?	Diplococcus present
184	E.A.W.	1½	F.	+	+	...	+	+	—	...	D.	3½ "	
185	T.E.	13	F.	+	+	...	+	+	—	...	D.	7½ "	Vomiting to coma, no convulsions
186	M.B.	4	F.	Through out	+	...	+	+	—	...	D.	11 "	Diplococcus seen, but no growth.
187	B.M.	1½	F.	Through out	—	...	+	+	—	...	D.	?	Symptoms of Vomiting Sickness
188	E.A.	3	F.	—	—	...	+	+	—	...	D.	8 "	Diplococcus present
189	T.J.	12	M.	+	—	...	D.	?	Reported as Vomiting Sickness, ? Why, see text
190	T.T.	2½	M.	+	+	...	+	+	—	...	D.	?	Diplococcus present, but no history sent
191	J.S.	30	F.	...	+	+	+	...	+	99°	+	—	...	D.	1½ hours	'Vomiting Sickness, without vomiting'
192	B.T.	4	F.	+	+	...	+	+	—	...	R.	...	
193	J.T.	5	F.	+	+	...	+	+	—	...	R.	...	
194	J.E.	6	F.	+	+	...	+	+	—	...	D.	24 hours	'No definite convulsions'
195	M.A.W.	3½	F.	+	+	...	+	+	—	...	D.	22½ "	No specimens sent. See text
196	M.R.	7	F.	+	+	...	+	+	—	...	D.	14½ "	
197	J.E.	6½	F.	+	+	...	+	+	—	...	D.	2 "	History incomplete
198	E.E.	20	F.	+	+	...	+	+	—	...	D.	24 "	No vomiting

TABLE V.—continued.

No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	DIAGNOSIS				Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated						Vomiting Sickness	V.S.	Meningitis	M			
199	D.A.	7	F.	...	+	++	++	...	+	—	—	—	D.	15 hours	No history of vomiting
200	A.B.	5	M.	+	—	—	—	D.	6 "	Diplococcus present
201	E.P.	27	F.	+	—	—	—	D.	9 "	History incomplete
202	F.E.	3	M.	+	—	—	—	D.	2 "	Diplococcus present
203	A.T.	6	M.	+	—	—	—	D.	48 "	Diplococcus present
204	B. ...	2½	+	—	—	—	D.	?	Reported as Vomiting Sickness.
205	T.E.S.	4	F.	+	—	—	—	D.	8 hours	Details lost
206	C.W.	30	F.	+	—	—	—	D.	12 "	Diplococcus present
207	M.C.	1½	F.	102°	+	—	—	—	D.	2 days	Diplococcus present
208	W.D.	2½	M.	?	+	—	—	—	D.	?	Diplococcus present, history incomplete
209	C.W.	12	M.	+	+	—	—	—	D.	2½ days	Diplococcus present, history incomplete
210	M.G.	7	F.	+	+	—	—	—	D.	6 hours	Diplococcus present, history incomplete
211	E.G.	10	F.	+	+	—	—	—	D.	9 "	Diplococcus present
212	E.H.	9	F.	+	—	?	+	—	—	—	D.	30 "	Diplococcus present
213	E.M.	1½	F.	+	+	—	—	—	D.	3 "	Diplococcus present
214	R.A.L.	9	F.	+	+	—	—	—	D.	6 "	Vomiting not stated, reported as Vomiting Sickness
215	C.T.	2½	M.	+	+	+	—	—	—	R.	...	Symptoms of M., but no specimens sent
216	N.W.	7	F.	+	+	—	—	—	D.	?	Diplococcus present
217	D.H.	3	M.	+	+	—	—	—	D.	2 hours	Diplococcus present
218	C.G.	6	M.	+	+	—	—	—	D.	16 "	Diplococcus present
219	C.W.	1½	M.	+	+	—	—	—	D.	1 hour	Diplococcus present
220	P.H.	2½	M.	+	+	—	—	—	D.	16 hours	Diplococcus present. See text
221	L.S.	4	F.	+	+	—	—	—	D.	11 "	History incomplete
222	C.C.	6	M.	+	+	—	—	—	D.	12 "	Diplococcus present. Preceding Catarrh
223	L.C.	3	F.	+	+	—	—	—	D.	6½ "	No convulsions seen
224	K.R.	1½	M.	+	+	—	—	—	D.	?	Diplococcus present
225	L.R.	40	F.	+	—	+	+	—	—	—	D.	1 week	Diplococcus present
226	H.W.	4	M.	+	+	—	—	—	D.	12 hours	Very acute; other symptoms not noted
227	J.D.	2	M.	+	+	—	—	—	D.	2 "	
228	J.T.	4	M.	+	+	—	—	—	D.	4 "	
229	G.J.	4	M.	+	+	—	—	—	D.	1 hour	
230	I.S.	3	F.	+	+	—	—	—	D.	6 hours	

TABLE V.—continued.

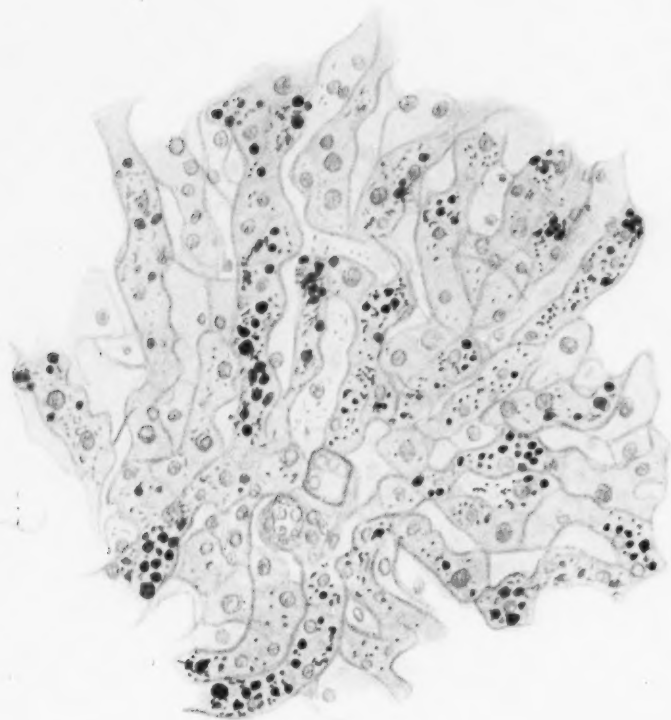
No.	Initials	Age	Sex	PAIN		VOMITING			Convulsions	Kernig's Sign	Rigidity of Neck-Muscle	Coma	Fever	Vomiting Sickness	DIAGNOSIS			Result	Duration	REMARKS
				Head	Abdomen	Initial	Secondary	Time not stated							V.S.	Meningitis	M			
231	I.R.	4½	F.	..	+	++	+	..	+	D.	14 hours	? Vomiting; 6 hours interval
232	M.H.	10	F.	..	+	+	?	+	D.	29 "	"
233	E.D.	4	F.	..	+	+	..	+	D.	7 "	"
234	S.M.	12	F.	..	+	+	..	+	D.	8 "	"
235	D.G.	6	F.	..	+	+	..	+	D.	33 "	"
236	N.R.D.	10	M.	..	+	+	..	+	D.	19 "	"
237	M.C.	13	F.	..	+	+	..	+	D.	34 "	"
238	R.C.	26	F.	..	+	+	..	+	R.	...	"
239	J.J.	65	F.	..	+	+	..	+	R.	...	"
240	B.J.	21	F.	..	+	+	..	+	R.	...	"
241	G.G.	8	M.	+	..	+	D.	11 hours	"
242	D.G.	11	F.	+	..	+	D.	7 "	"
243	W.G.	9	M.	+	..	+	R.	...	"
244	A.G.	4	F.	+	..	+	R.	...	"
245	F.W.	9	F.	+	..	+	D.	? 18 hours	"
246	H.W.	1½	M.	+	..	+	D.	2 "	"
247	P.C.	25	F.	..	+	+	..	+	R.	...	"
248	W.B.H.	5	M.	..	+	+	..	+	D.	4 hours	"
249	G.S.H.	2½	M.	+	..	+	D.	5 "	"
250	A.M.H.	8	F.	+	..	+	D.	8 "	"
251	A.H.	42	F.	+	..	+	R.	10½ hours	"
252	J.McB.	6½	M.	+	..	+	D.	...	"
253	L.R.	26	F.	+	..	+	D.	48 hours	"
254	B.R.	14½	F.	+	..	+	D.	4 "	"
255	R.M.	4	F.	+	..	+	D.	25 "	"
256	C.S.	25	F.	+	..	+	D.	8 "	"
257	E.B.	1½	F.	+	101.4°	+	D.	44 "	"
258	C.W.	8	F.	+	101.0°	+	R.	...	"
259	S.D.	6	F.	+	..	+	D.	21 hours	"
260	J.F.	4	F.	..	+	+	..	+	D.	19 "	"
261	C.Y.	10	F.	..	+	+	..	+	R.	...	"
262	E.M.	25	F.	..	+	+	..	+	R.	...	"
263	P.M.	6	F.	..	+	+	..	+	R.	...	"
264	P.R.	8	M.	..	+	+	..	+	D.	20 hours	"
265	A.R.	2½	F.	..	+	+	..	+	D.	4 "	"

EXPLANATION OF PLATES

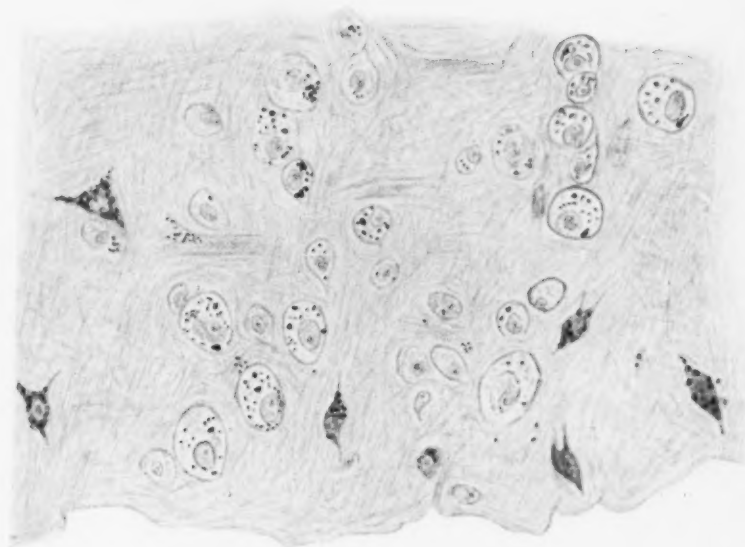
PLATE I

Drawings reduced to $\frac{3}{4}$ original size for purposes of reproduction.

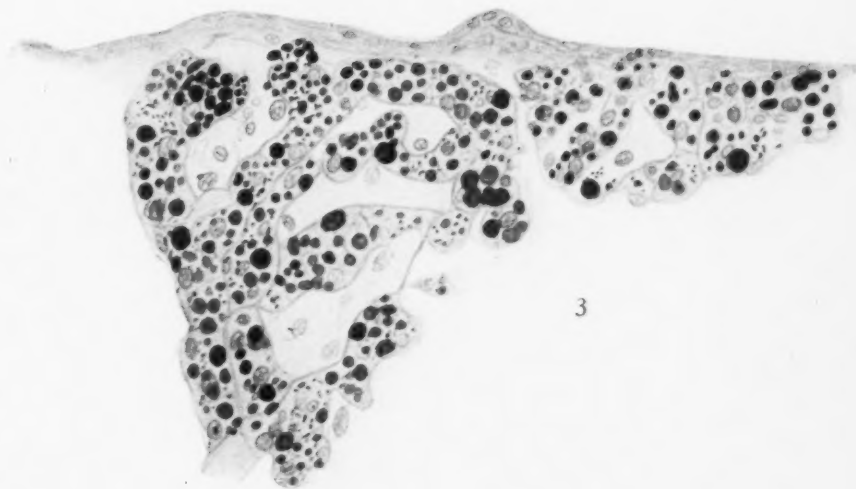
- Fig. 1. Cerebrum of dog, after ingestion of ackee extract. Showing minute fat droplets in nerve cells and capillary. Flemming's fixative and safranin stain. 4 mm. objective, comp. oc. 4, tube 150.
- Fig. 2. Kitten 3. Liver, after ingestion of ackee extract. Showing fatty metamorphosis, less advanced stage than fig. 3. Flemming and safranin. 4 mm. obj., oc. 4, tube 150.
- Fig. 3. Kitten 2. Liver, after ingestion of ackee extract. More advanced fatty degeneration than in Kitten 3 (see last fig.). Flemming and safranin. 4 mm. obj., oc. 4, tube 160.
- Fig. 4. Kitten 2. Kidney, after ingestion of ackee extract. Fatty degeneration of ascending limbs of Henle's loop, and of convoluted tubule. Flemming and safranin. 4 mm. obj., oc. 4, tube 160.
- Fig. 5. Kitten 2. Kidney, after ingestion of ackee extract. Many tubules have lost epithelium and contain blood, apparent increase of interstitial tissue in consequence of disappearance of epithelium. Stained haematoxylin and Hansen's modification of Van Gieson. 4 mm. obj., oc. 4, tube 158.



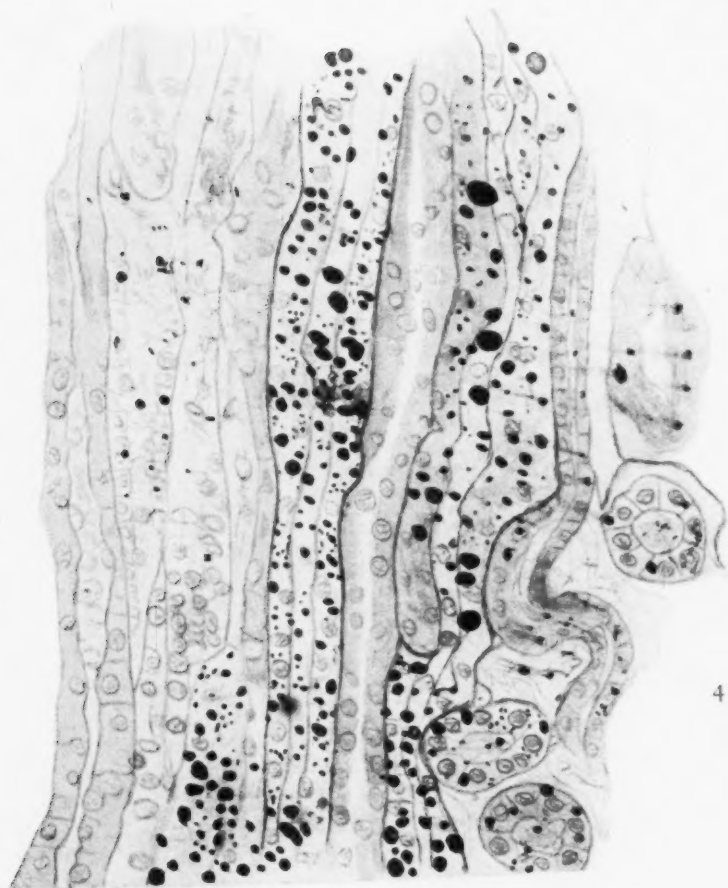
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3



4



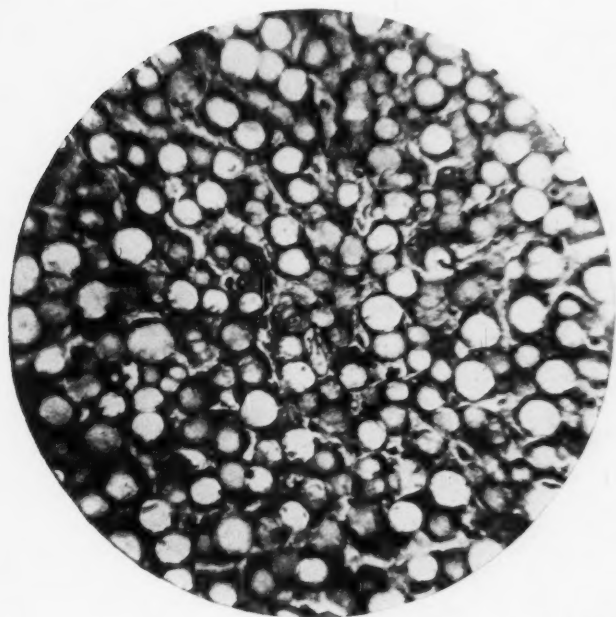
5

PATHOLOGY OF VOMITING SICKNESS.

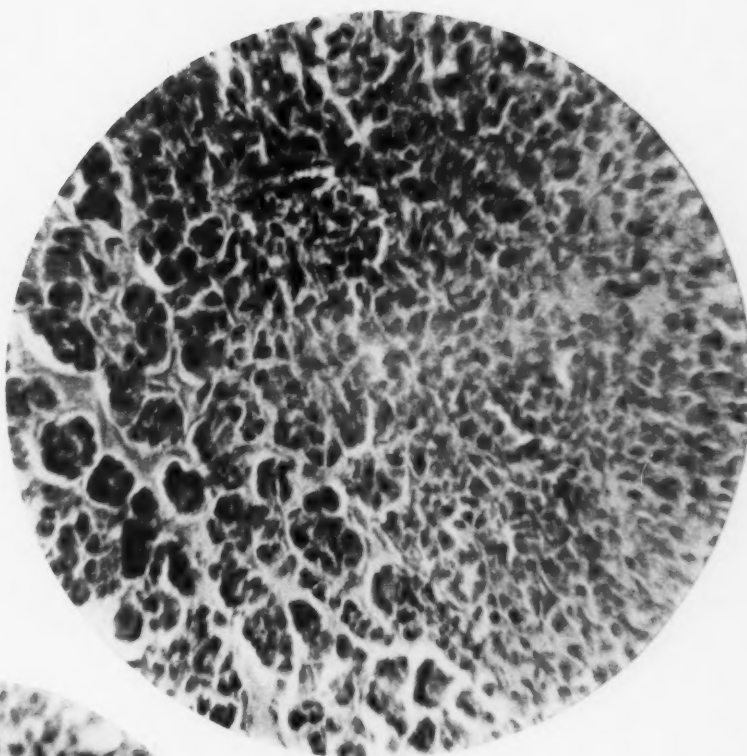
PLATE II

Micro-photographs reduced to $\frac{3}{4}$ original size for purposes of reproduction.

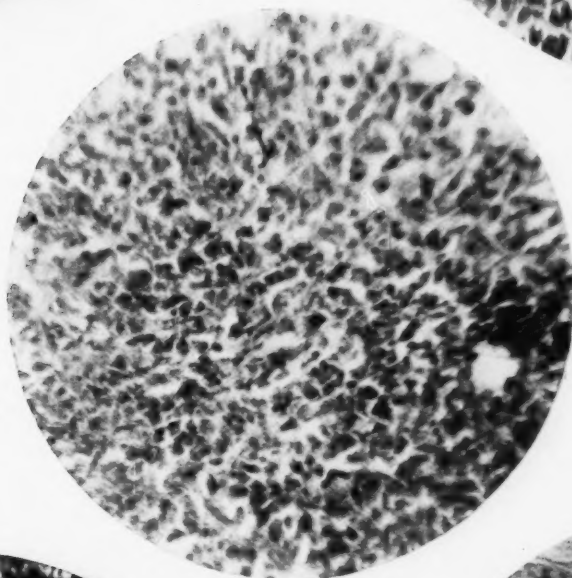
- Fig. 1. Liver from the case of ackee poisoning, M.S., described in the text, showing intense fatty metamorphosis. This should be compared with Seidelin's report, Pl. XXXII, fig. 3. The change is even more marked in the case of M.S., because it is the result of repeated small doses of the poison, with a fulminating termination.
- Fig. 2. Pancreas from M.S. (ackee poisoning) showing a few acini with recognisable epithelium, but marked necrobiosis and fatty changes elsewhere.
- Fig. 3. Liver from E.W., a case of human vomiting sickness, showing fatty changes and necrobiosis. The condition here is intermediate between that of the kitten, fig. 4, and the intense affection of the ackee poisoning case, M.S., fig. 1.
- Fig. 4. Kidney from same patient, E.W., showing marked epithelial necrobiosis. It is even more marked than in Seidelin's case (Pl. XXXIII, fig. 4, of his report).
- Fig. 5. Pancreas from L.D., another case of vomiting sickness, showing necrobiosis and fatty change almost universally distributed.



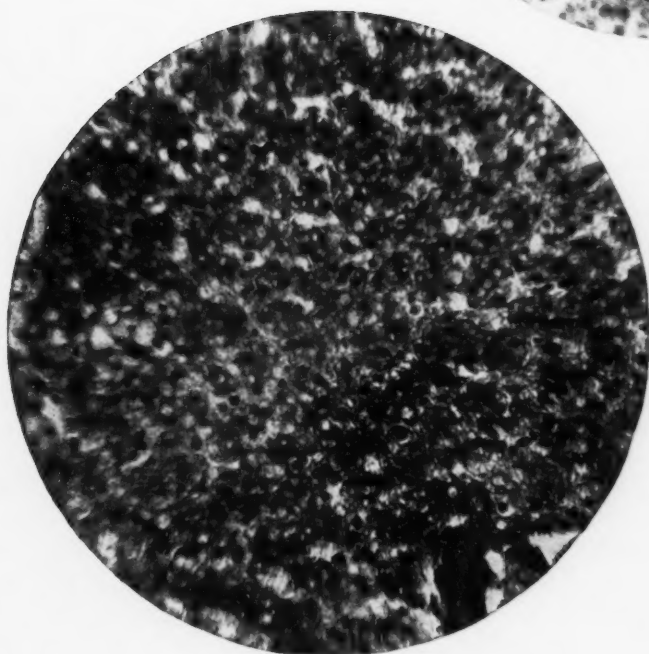
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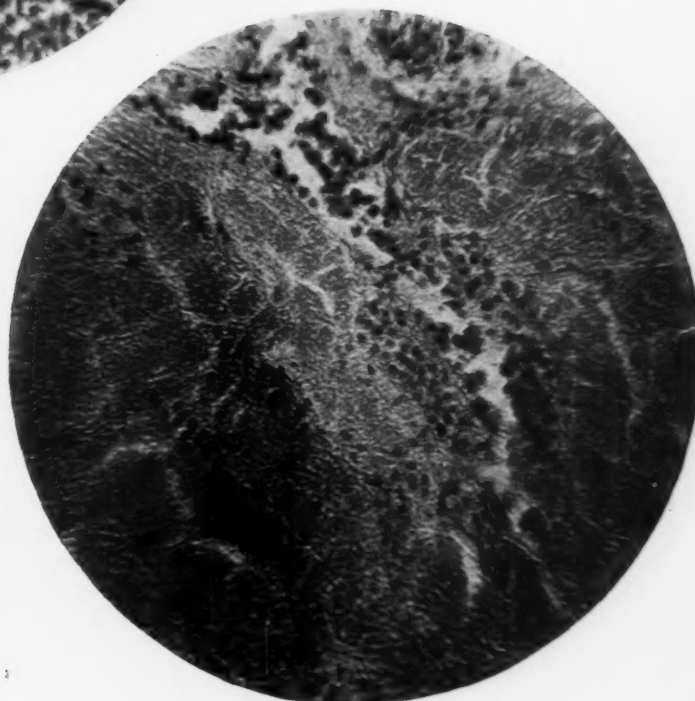
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5



3



4

PATHOLOGY OF VOMITING SICKNESS.

ANKYLOSTOMIASIS : SIMPLIFIED DIAGNOSIS AND TREATMENT

BY

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INTRODUCTION

This paper is written in order to put on record a simple method which has been found of great help in carrying out the treatment of ankylostomiasis. Those who have had any experience with this disease will be familiar with the difficulty of determining whether, after one treatment has been given and many worms have been expelled, any further anthelmintic treatment is necessary. The method about to be described has been adopted with a view to solving this difficulty. Several methods are already in use for this purpose, i.e., for determining whether an individual is or is not still harbouring parasites; and these will be considered before the new test is described. In conclusion, an outline will be given of the procedure followed in the Swatow Mission Hospital in treating patients suffering from ankylostomiasis.

METHODS THAT ARE AT PRESENT IN USE TO DETERMINE WHETHER AN INDIVIDUAL IS OR IS NOT HARBOURING PARASITES

These may be divided into two classes, viz., (i) those depending on the results of a microscopic examination of the faeces, and (ii) those depending on the results obtained by the use of anthelmintics.

(i) Of methods depending on the results of microscopical examination for ova, three are in common use :—

(a) Specimens may be prepared in the ordinary way by mixing a fragment of faecal matter with a drop or two of water on a microscope slide and applying a coverslip. If this plan is followed the examination of fourteen slides is necessary before an individual can be pronounced free from infection (Clayton Lane, 1915).

(b) The specimen for the microscope may be prepared from the deposit that is left after a specimen of the faeces has been thrice shaken up in clean water and centrifuged (Billings, 1915).

(c) The specimen may be prepared from the scum that comes to the surface when the faecal residuum obtained by centrifuging with water and then with weak calcium chloride solution is finally centrifuged with a solution of calcium chloride of a specific gravity equal to 1.250 (Bass, 1909).

If either of these two latter plans is followed the examination of three slides should be sufficient.

(ii) Of the methods depending on the results obtained by the administration of anthelmintics there are also three that have found favour from time to time:—

(a) One may order a new course of treatment so often as the previous treatment has resulted in the expulsion of ankylostomes. [NOTE.—In this case one unnecessary treatment is always given to every patient.]

(b) One may calculate from the results of the first treatment the number of courses of treatment that will be required. Thus Leys (1912), using ninety grains of thymol divided into three doses, formulated the following rule:—‘If 150 parasites or more are passed, three more courses are likely to be required; if 50 parasites or more, two more courses are required; if less than 50 parasites, then one more course is necessary.’

(c) Assuming that the effectiveness of certain anthelmintics is fairly constant (e.g. that 60 grains of thymol will remove 83 per cent. of the parasites present, while 30 grains of beta-naphthol will only remove 68 per cent.), it may be calculated that the number of worms left, even in a large infection, after two treatments by thymol, will be negligible, whereas it will require three treatments with beta-naphthol to produce the same result (Clayton Lane, 1915).

A brief consideration of these methods shows that all those in which the microscope is used to look for ova are tedious. They

do not exclude the possibility of male worms or immature worms being present, while the microscopic methods are either based on purely theoretical considerations or else depend on the observations made by a nurse—who in many cases is a 'native' and may not have been very careful.

It is obvious that there is room for

A SIMPLE AND RELIABLE TEST FOR DETERMINING THE PRESENCE OF ANKYLOSTOMES

The test suggested depends upon two facts:—(i) a trace of blood is constantly present in the faeces of ankylostomiasis patients, and (ii) there is a test for blood so sensitive as to reveal its presence in a dilution of 1 in 800,000.

The test is both simple and rapid, for the only reagents employed are the test solution and hydrogen peroxide, and half a dozen specimens can be examined in ten minutes. The reagent is prepared as follows:—100 c.c. of a 20 per cent. solution of caustic soda treated with 2 grammes of phenolphthalein and 10 grammes of zinc dust. The bright rose-coloured solution is heated gradually until it has assumed a slightly yellowish tone. The supernatant fluid is poured off into a coloured glass bottle and the access of air is prevented by the addition of a little liquid paraffin which floats on the top. The test is made as follows:—To 2 c.c. of a watery solution of the faeces is added 1 c.c. of the alkaline solution of phenolphthalin (i.e., reduced, and therefore colourless, phenolphthalein) and then 1 drop of hydrogen peroxide. In the presence of blood some of the phenolphthalin is re-oxidised to phenolphthalein and a bright red colour appears.

It is necessary to make sure, before applying the test, that the patients are not suffering from peptic ulcers, haemorrhoids or other gross lesion causing bleeding into the gastro-intestinal tract, and that they have not eaten meat* or blood during the previous 72 hours. In actual practice these points have not caused any real difficulty.

* This includes cockles which are a favourite condiment in Swatow and are often eaten in sufficient quantities to give rise to blood reaction in the faeces.

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This test for occult blood is found in modern text-books (e.g. Simon, 1913), and the presence of blood in the faeces of ankylostome patients is one of the most rudimentary facts in connexion with this disease, so the writer can make no claim to originality; but he wishes to draw attention to this application of the test because it has so greatly simplified the treatment of ankylostomiasis patients.

In order to show how this test is used in our practice it may be well to give

AN OUTLINE OF THE PROCEDURE FOLLOWED IN TREATING PATIENTS SUFFERING FROM ANKYLOSTOMIASIS

A patient who presents himself at the out-patient department suffering from one or more of the symptoms which are commonly present in ankylostomiasis (e.g. anaemia, dyspepsia, giddiness, breathlessness, aching, etc.) is told to bring a specimen of his faeces on the first convenient occasion.

Generally the infection is so severe that ankylostome ova are found under the microscope in the first slide prepared from this specimen; but if this is not so, the patient is told to exclude meat and blood from his diet for three days and then bring a specimen again. If blood is found in this second specimen, or if eggs were present in the first, the patient is at once admitted for treatment.

The patient takes a light meal about 5 o'clock and at 7 o'clock is given a powder containing:—

Calomel	grs. III.
Phenolphthalein	grs. II.
Santonin	grs. IV.

The following morning no food is allowed, and at 6 and at 8 he is given 40 grains of beta-naphthol suspended in peppermint water by mucilage of tragacanth. At 10 a.m. 30 grains of thymol* is administered in capsules, and at 12 noon a draught of magnesium sulphate (from six drachms to one ounce) in hot water. If the bowels do not move before 3 p.m. the dose of saline is repeated then.

* This combination of drugs (Beta-naphthol 80 grains and Thymol 40 grains) has proved more efficient than 120 grains of Beta-naphthol, and causes less anxiety than the administration of 90 grains of Thymol. If the latter drug is administered in so large a dose, any delay in securing free movement of the bowels must cause considerable anxiety, since the most efficient purgative in the pharmacopoeia—castor oil—is contra-indicated, and if the Thymol is not evacuated soon, it may be absorbed in sufficient quantity to cause fatal cardiac weakness.

As a rule the patient chooses to go home that evening, and presents himself at hospital on the next out-patient day.

If more than 250 ankylostomes have been expelled at the first treatment it is certain that a second treatment will be required; this may take place about seven days after the first. It is carried out in the same manner as the first, with the exception that no santonin is given in the evening powder.

If the first treatment has produced a smaller number than 250, then the patient is told, about a week after the first treatment, to refrain from all meat and blood for three days and to bring a specimen of his faeces at the end of that period. No time is spent in examining this for ova, but it is at once tested for blood, and if this is present, a second treatment is ordered—to be carried out as described above.

About ten days later a specimen is again brought (after three days' abstention from blood and meats), and if blood is still present, treatment is again repeated.

Thus the cycle consists of four parts:—

- (1) Anthelmintic treatment.
 - (2) A week of ordinary diet.
 - (3) Three days' abstention from blood and meat.
 - (4) An examination of the faeces for blood, going back to
- (1) Anthelmintic treatment, as often as blood is found in the faeces.

This routine is followed till blood is no longer present in the faeces, and so the cycle is broken. As soon as this happens the anthelmintic part of the treatment is at an end, and attention may be concentrated upon building up the patient's impoverished blood. For this purpose we find nothing more effective than freshly prepared Bland's pills, of which we use about 3,000 to 4,000 a week.

Altogether the test has been applied 1,636 times in the faeces of 500 ankylostome patients during the last twelve months. Of the last 100 patients who have completed their treatment (i.e. have remained under observation till their faeces were free from blood):—

- 50 cases required only one treatment,
- 30 cases required two treatments.
- 12 cases required three treatments,
- 6 cases required four treatments,
- 1 case required six treatments, and
- 1 case required eight treatments.

The sensitiveness of the test is shown by the fact that often less than twenty worms were expelled in the last treatment the patient received, i.e. so small a number of ankylostomes were able to give rise to an amount of blood sufficient to be detected by this test.*

For immigration officers and others who require an absolute guarantee that not one female worm is present in the intestine, this test may be deemed inadequate, for a few worms may be present in an individual whose faeces contain no blood. However, if it is first applied (with the proper safeguards) to all those who are to be examined, the troublesome microscopical examination of the faeces instead of being required for all the individuals will only be necessary in the case of those whose stools show no signs of blood. The amount of time thus saved will be considerable.

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* Possibly a fallacy underlies this. It is well known that the wounds made by ankylostomes in the intestinal mucosa may continue to bleed for some time after the worms have been removed. It may be expected that this haemorrhage will have ceased within a week and will not give rise to the presence of blood in the specimen of faeces examined ten days after the ankylostomes have been expelled. It may be that in some cases either a pyogenic infection has taken place, or an ulcerative process has been set up, or the patient is so debilitated that there is delay in the healing of the ankylostome wounds, and so blood is found in the faeces although parasites are no longer present in the intestine.

THE VALUE OF DIFFERENTIAL BLOOD COUNTS IN THE DIAGNOSIS OF MALARIA

BY

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I. INTRODUCTION

After a considerable amount of work in the making of differential blood counts both in health and disease—and especially in malaria—I came to the following main conclusions:—

- (1) That the increase of large mononuclear leucocytes found in malarial bloods is a fairly persistent change;
- (2) that this change of ratio, evidenced by a differential count, is sufficiently specific to form a reliable test of infection; and
- (3) that the phenomenon persists after the time when parasites disappear from the blood-stream (i.e. when parasites are no longer discoverable by practical microscopy), and so permits of a post-febrile diagnosis being made.

The above three assertions, in addition to subsidiary points, are elaborated below. The observations were conducted over a period extending from October, 1912, to July, 1914. The majority were carried out under conditions eminently suitable for the elimination of error—points which will be dealt with later.

II. NECESSARY DEFINITIONS

Owing to the confusion which still exists in the identification and classification of blood cells, it is well to define first the standards and method adopted by the writer.

(a) *Cell Constituents of the Blood*

The following blood cells, excluding those of rare diseases, are

well known:—Polymorphonuclear Leucocyte, Eosinophile Leucocyte, Large Mononuclear Leucocyte, Large Lymphocyte, Small Lymphocyte, Mast Cell, Transitional Cell, Endothelial Cell, Broken Nuclei.

No observer, as far as I know, gives the results of differential blood counts with such elaborate tabulation. Were this so, a common basis for comparisons would be found.

(b) *My Grouping of the Cells*

To disregard or incorporate under another heading any of the above cells is obviously a faulty proceeding. The error will be small, however, if the cell—omitted or misnamed—is a rare constituent of the blood.

At first, unfortunately, I omitted to enumerate broken nuclei, and so an error exists in the earlier counts. I hope to show, however, that this omission in no way prejudices the inferences to be drawn from these counts. Later, a separate class was assigned to them.

The transitional cell, as it appears to behave like the large mononuclear, has been incorporated with the latter.

The endothelial cell, very rarely seen, has also been enumerated with the large mononuclears.

My method of classification of these two rare cells is probably little different from that of other observers. Assuming this to be the case, we still find difficulties in comparing counts, owing to the divergent views on the classification of the large mononuclear leucocyte and the large lymphocyte. This divergence is so important, and affects, as I hope to show, the value of the count so materially, that a detailed description of these cells is given now.

Large Lymphocyte

The nucleus is circular or somewhat square. The shade of stain acquired with Leishman's is from deep blue to purple, but not pink or violet. The colour is not so deep as in the older or more compact nucleus of the small lymphocyte. The nucleus fills from one-third to one-half of the cell, and the protoplasm also takes on a bluish tinge. The latter is either clear or hazy, as if filled with semi-transparent jelly. It may or may not contain azurophile granules.

Though actual size is a matter of how the slide is prepared, yet relative size must be the factor in dividing the small from the large lymphocyte.

In the bulk of my counts the standard of differentiation between these two cells was made as follows:—Any lymphocyte of the size of, or larger than, the average polymorph in the film was assigned to the large lymphocyte class, and all lymphocytes smaller than the polymorphonuclear to the small lymphocyte class. In a few earlier counts the standard was different. (*Vide* Paragraph 5 (a).)

Large Mononuclear Leucocyte

The following essential characteristics were seen before any cell was placed in this class (as most of the distinctions are relative, a rapid survey of the slide is advisable, when relative sizes and depths of staining can be registered mentally):—

- (1) The cell must be as big as the largest polymorphonuclear seen. The size of the large mononuclear is almost invariably, of course, much larger than this standard. The envelope is usually of irregular shape.
- (2) The nucleus must show less depth of stain than the nucleus of any other white cell in the film (excluding the mast cell when the nucleus of this fails to stain). Usually, with a stain working well, it is of a cherry-violet colour, as opposed to the bluish purple of the large lymphocyte.
- (3) The nucleus is woolly in appearance, sometimes ovoid (true large mononuclear), sometimes kidney-shaped (transitional cell), and very rarely squarish (endothelial cell). It is never circular.

Other normal characteristics are:—

- (4) Presence of chromatin granules. These are usually triangular chips, but may appear as dots, and their total numbers rarely exceed ten. Absence of these in a typical cell with the above three characteristics does not, with me, debar the cell from this class, as the granules may be obscured from vision by a superimposed nucleus.
- (5) The protoplasm is usually clear and non-staining, or faintly blue. Often the envelope is ruptured and the protoplasm lies scattered, but the envelope remains apparent as an almost complete cell.

Absence of any of the three essential characteristics led me to place the cell in the large lymphocyte class.

Characteristic in Malaria:

- (6) Pigment is often observable, lying within the protoplasm. This might be confused with superimposed dirt, which, however, is avoided by careful staining methods.

(c) *Method of Staining*

Leishman's stain, formula, and method were used up to the point of application to the film. From this point I carry the staining to a state of overstain and consequent deposit. The stain is then poured off and, with the slide held under a tap, two drops of pure alcohol are made to run down the surface. Immediately these have reached the bottom, the slide is put in the water stream. The slides are only just washed and then are blotted dry, the drying being hastened by gentle warming if necessary, otherwise uneven coloration results. This finishing method avoids the use of distilled water for washing, and the light wash of alcohol on an overstained slide does not decolorize too much, but, on the contrary, gives a perfect pink colour to the red blood corpuscles and ensures a complete absence of dirt.

(d) *Method of Counting from the Smear*

All counts were made from any part of the smear, as I have never found any preponderance of a particular cell in any special area.

The number of leucocytes counted, where not stated in the Tables, was any round number from 100 to 800.

III. CERTAIN ERRORS. HOW AVOIDED

(a) *Error of Nomenclature*

To ensure uniformity each cell must be identified correctly and the same classification be adopted throughout. Such uniformity exists in my counts with the two slight exceptions already mentioned; i.e. the omission of broken nuclei and a different standard of differentiation between the large and small lymphocytes in the earliest counts.

(b) *Error of Bias*

In order to avoid any possible objection of trying to prove a preconceived theory, I made a point of having nearly every slide numbered only, the suspected disease—if any—man's name, etc., being kept secret by my assistant until the count was made. More

especially, in order to avoid bias, slides of healthy men or of men with different ailments were intermingled indiscriminately with the malarial bloods. This was not always possible, as, occasionally, only one slide would come up for examination.

(c) *Error of Technique*

In part this error was eliminated by the stain being prepared, the slide stained and the count made by one person only. My assistant made every blood smear. The error of technique, despite these precautions, may still be large, as a little experience soon showed me that counts from the same patient may vary according to the way the smear is made.

This phenomenon was almost entirely confined to malarial bloods in which my count was disturbed by the presence of numerous broken nuclei. When this first occurred the question arose as to whether these nuclei could be ignored.

With a little consideration it is evident that there is only one conceivable circumstance in which these nuclei can be disregarded. This circumstance will be if the nuclei originate from all classes of leucocytes, and then only if their numbers are in the same proportion as their parent cells. Let us suppose a simple case where a count gives:—

Polymorphonuclear	60 per cent.
Large mononuclear	10 „
Lymphocyte	10 „
Nuclei	20 „

If the broken nuclei are ignored, we shall have counted 80 cells in the proportion of 60 : 10 : 10, and 100 complete cells will probably be in the proportion of 75 : 12·5 : 12·5. If the nuclei originated, in this example, from all three kinds of cells observed, then the same proportion might be arrived at by neglecting to count them, but if they originate from one class of cell only (say the large mononuclear), the count in reality will be polymorphonuclear 60 per cent., large mononuclear 30 per cent., and lymphocytes 10 per cent.

That all classes of leucocytes do not give rise to broken nuclei was evident, as I had already observed that they figured rarely in healthy bloods and commonly in malarial bloods, in which the concomitant change is one of increase of large mononuclears. I therefore made a separate entry for broken nuclei in future.

The presence of broken nuclei in excess is to be looked upon as an error of technique. I satisfied myself upon this point by experimenting upon a malarial blood, making films by rough and gentle methods. Those films prepared carefully by moving the blood uphill in the rear of the spreader gave the least number of broken nuclei, and, incidentally, the greatest proportion of large mononuclears.

(d) *Error of Insufficient Count*

For great accuracy it is said that at least 2,000 leucocytes should be enumerated. Time never allowed of this. In my later counts I fixed 400 as a minimum. This error, which could not be eliminated, should not influence the comparative value of the tables, although the positive value of any one table, taken alone, will be affected.

(e) *Error of Insufficient Sampling*

For still further accuracy a larger sample than one drop should be taken at one time, and, moreover, frequent sampling should be made throughout the twenty-four hours. Such a labour is almost beyond the capacity of one individual

In one respect my slides were nearly all uniform, inasmuch as they were taken in the early morning before digestive leucocytosis sets in.

(f) *Mathematical Errors*

Of the four principal methods of striking averages, the least reliable is used in my tables, i.e. the Arithmetic method. If another (say the Geometric) method is used, it will still be found that the averages exhibit the same relation to each other as is found by using the former method.

Mathematically it is true that averages need correction. As the great part of this paper deals with the difference between the average number of large mononuclears found in health and the number found in malaria, it will be advantageous to dismiss here the only argument which an actuary could apparently bring to upset the value of my findings.

If 5 is the average number of large mononuclears found in a healthy blood, and 400 cells were counted, an actuary can show by Poisson's formula that the number might be as *high* as

$\cdot 05 + \cdot 03 = 8$ per cent. Again if 15 is the average in a malarial blood, and 400 cells were counted, the number might be as *low* as $\cdot 15 - \cdot 05 = 10$ per cent. In this event comparisons between a healthy and a malarious slide would be worthless. But we deal here with a large series of counts, and it would be absurd to argue that each individual healthy count was too low, and each malarial count too high.

IV. FIRST MALARIAL OBSERVATIONS

(a) *Station non-malarious*

The military cantonment, situated fifteen miles from Darjeeling, in which I became stationed early in October, 1912, offered ideal conditions under which to carry out the following observations, inasmuch as it was entirely free from Anopheline mosquitos and therefore non-malarious.

The barracks ranged from 4,500 to 5,500 feet above sea-level, and were built upon the side of a very steep mountain. Thus, though the rainfall was on the average 175 inches during the year, all rainwater quickly drained away.

The nearest districts where Anophelines prevailed and malaria was indigenous were at lower levels, and were rarely, if ever, frequented by the men of the battalion.

Personal search for adults and larvae of Anophelines invariably failed to give positive results; mosquito nets were never used, and the local reputation of the cantonment was that it was quite free from mosquitos.

The best proof that men of the regiment never contracted malaria whilst present with the battalion in this station is that the admissions for this complaint for the whole of 1912 and up to November, 1913, were made up of—

- (1) Cases attacked whilst on duty in the Tista Valley.
- (2) Cases beginning with a primary attack or relapsing immediately on return from furlough or on being newly recruited.
- (3) Relapses of attacks already under observation, originating as either (1) or (2).

Thus if malaria were at all indigenous or had been contracted by the men during their off-duty times, there would necessarily have been cases which would not fall into any of the above groups. There were none.

(b) *Epidemic. 59 Cases of 'Fever'*

On my arrival in this station, the battalion—a Gurkha one recruited mainly from south-eastern Nepal—was split up into numerous detachments; the majority of the men were in cantonments, whilst the remainder were posted in companies and half-companies at intervals along the Tista Valley as far as the Thibet frontier.

Upon the return of the outposts, 59 cases of 'fever' were admitted into hospital, a figure representing nearly 25 per cent. of the men thus employed.

(c) *Quinine Test*

Other duties were so pressing that I had no time for thorough examination of each case on the day of admission. The first main procedure which I carried out, whilst at the same time commencing the task of taking cases in detail, was to divide the wards longitudinally and to treat empirically one side with quinine and the other side with diaphoretics only.

Results became apparent very shortly. On the quinine side, some cases responded at once to large doses (30 grains quinine per diem), others made no improvement whatsoever. On the other side, where diaphoretics were employed, the same state of affairs in time was noticed, but with this difference: the cases which showed decline of temperature to normal had no marked signs of malaria, e.g., enlargement of spleen, typical chart or the classical symptoms of this disease; whilst the cases uninfluenced by diaphoretics proved themselves to be malaria by typical intermittent periods.

(d) *Parasite Test*

Meanwhile blood examination was progressing, and results both positive and negative were obtained. It should here be noted that certain cases passed into convalescence without a diagnosis or a blood examination being made.

(e) *Grouping by above Tests*

Omitting clinical histories and tentative diagnoses obtained by examination at the bedside, the cases, by the above simple tests, arranged themselves as follows:—

- A. Cases reacting to quinine and not to diaphoretics:—
 Charts typical ... *a.* Blood +, *b.* Blood −.
 Charts atypical ... *a.* Blood +, *b.* Blood −.
- B. Cases not reacting to quinine but to diaphoretics:—
 Charts atypical ... Blood −.
- C. Residuum of cases with notes incomplete, owing to convalescence.

Numerically the cases divided thus:—

- 19 cases with parasites present, and generally typical charts.
- 40 cases without parasites in blood, with a rough division possible from the appearance of the charts.

When treating cases empirically with quinine, I felt that no great stress could be laid upon 'apparent' reaction to the drug, as there must, by the laws of chance, be some cases whose fever would normally terminate on the day when quinine was started. Diagnosis could be accurately established in the case of 19 men. The remaining 40 cases presented an interesting problem. After a study of the notes on these cases and the clinical signs and symptoms, I was left with an open mind, though, in the bulk of the cases, the evidence was against malaria.

(f) *Results of Differential Counts*

Despite the convalescence of many of the cases, it occurred to me that a differential blood count might help me. I therefore began a systematic count of the blood of every one of these patients.

An increased percentage of large mononuclears is such a well-known change in malaria that the counts in the 19 cases of positive malaria, where parasites were found, called for no surprise.

For the sake of clearness I give these large mononuclear percentages in a separate table below, although the full counts can be traced in Tables 10(*a*) and 11 under the same case numbers.

(NOTE.—*Serial Numbers* in all these Tables are merely progressive totals of counts made. Where the same *Serial Number* occurs twice, the same count appears against it. They are used to facilitate reference.

Case Numbers are used in place of patients' initials, and occur over and over again. They are used to allow case counts to be traced through successive weeks.)

TABLE I.—(19 cases, 19 counts.)

	Serial No.	Case No.	Large mono- nuclear percentage	Remarks
Day of attack	1	1	14.66	
"	2	2	10.00	
"	3	3	14.00	
"	4	4	26.00	
"	5	5	18.00	
"	6	6	15.00	
"	21	7	26.50	
"	22	8	8.00	<i>Vide</i> Serial No. 269, Table II
"	23	9	22.00	
"	24	10	19.00	
"	25	29	24.00	
4 days after	270	12	21.00	
18 "	283	13	16.00	
19 "	285	14	15.00	
20 "	286	15	20.00	
21 "	287	16	19.00	
22 "	289	17	19.00	
22 "	290	18	18.00	
23 "	291	19	3.00	<i>Vide</i> Serial Nos. 297 and 312

The remaining 40 cases are now shown purposely divided into Tables 2 and 3. The full counts of Table 2 can be studied in the larger Table 13 under the Serial Nos. 238-253.

TABLE 2.—(10 cases, 16 counts.)

	Serial No.	Case No.	Large mono-nuclear percentage	Remarks
Day of attack	238	11	20.50	
"	240	20	20.50	
4 days later	242	21	17.00	
4 "	243	22	13.00	
15 "	244	22	13.00	
17 "	245	23	15.00	
20 "	239	11	15.00	
23 "	246	24	8.00	<i>Vide Serial No. 246, Table 13</i>
32 "	247	25	23.00	
36 "	249	26	16.00	
40 "	251	27	14.00	
44 "	241	20	13.00	
48 "	253	28	16.00	
50 "	248	25	16.00	
63 "	252	27	15.00	
66 "	250	26	11.33	

NOTE.—Case No. 24 had a relative eosinophilia of 39.33 per cent. I also inferred a positive eosinophilia from the excess of leucocytes seen in the film. Assuming the positive eosinophilia to be 4,000 eosinophiles in 14,000 cells, in order to bring the polymorphonuclears to the healthy average found in a later table, the large mononuclears become 11.20 per cent. ; or again, if the increase were 5,000 eosinophiles in 15,000 cells, which brings the average of the polymorphonuclears to that found in malaria, the large mononuclears become 12 per cent. This method of 'reduction to normal' is amplified in Paragraph 5a.

Whether I was right or wrong either in assuming the eosinophilia to be due to an additional infection or in including this case in this group, I prefer to leave it there now, rather than omit cases to improve the appearance of this table.

TABLE 3.—(30 cases, 36 counts.)

DIFFERENTIAL BLOOD COUNTS IN CONVALESCENCE FROM 'DENGUE FEVER'

Serial No.	Case No.		Poly-morpho-nuclear	Eosino-phile	Mast cell	Large mono-nuclear	Large lympho-cyte	Small lympho-cyte
168	1De	1st week ...	51·00	—	—	9·00	—	40·00
169	2De	„ ...	65·00	10·00	—	3·00	—	22·00
170	3De	2nd week ...	58·00	13·00	2·00	5·00	14·00	8·00
171	4De	4th week ...	72·00	9·00	1·00	3·00	14·00	1·00
172	5De	„ ...	52·00	8·00	1·00	5·00	27·00	7·00
173	6De	5th week ...	40·00	15·00	—	3·00	23·00	19·00
174	7De	„ ...	54·00	18·00	1·00	4·00	4·00	19·00
175	8De	„ ...	41·00	19·00	—	6·00	9·00	25·00
176	9De	„ ...	65·00	7·00	—	3·00	6·00	20·00
177	10De	„ ...	50·00	11·00	—	2·00	10·00	27·00
178	11De	„ ...	57·00	12·00	1·00	2·00	13·00	16·00
179	12De	6th week ...	47·00	3·00	—	4·00	17·00	29·00
180	13De	„ ...	38·00	20·00	2·00	4·00	9·00	27·00
181	14De	„ ...	49·00	6·00	1·00	2·00	14·00	28·00
182	15De	„ ...	54·00	8·00	—	4·00	9·00	25·00
183	16De	„ ...	41·00	16·00	—	3·00	11·00	29·00
184	17De	„ ...	70·00	14·00	1·00	1·00	8·00	6·00
185	18De	7th week ...	54·00	12·00	2·00	5·00	10·00	17·00
186	19De	„ ...	45·00	9·00	1·00	2·00	9·00	34·00
187	20De	8th week ...	42·00	17·00	1·00	2·00	13·00	25·00
188	21De	„ ...	64·00	4·00	—	4·00	10·00	18·00
189	22De	„ ...	50·00	19·00	—	3·00	13·00	15·00
190	23De	„ ...	47·00	12·00	—	3·00	17·00	21·00
191	24De	„ ...	70·00	7·00	2·00	2·00	7·00	12·00
192	25De	„ ...	52·00	10·00	—	4·00	22·00	12·00
193	26De	„ ...	56·00	14·00	—	4·00	10·00	16·00
194	27De	„ ...	57·00	4·00	1·00	3·00	6·00	29·00
195	28De	„ ...	47·00	16·00	—	1·00	7·00	29·00
196	29De	9th week ...	68·00	2·00	—	—	8·00	22·00
197	30De	„ ...	52·00	12·00	1·00	2·00	9·00	24·00

(g) *Grouping by the Differential Count*

I think it will be admitted that these 39 cases, by the parasite search and differential count method, group themselves naturally as I have distributed them in Tables 1, 2 and 3.

A study of these three tables shows the following main characteristics :—

Table 1. (a) The positive malarias show a high percentage of large mononuclears.

(b) This increase is maintained up to the fourth week.

Table 2. (a) There is a similar increase of large mononuclears.

(b) This increase is maintained up to the ninth week.

Table 3. (a) The large mononuclears are not half so high as in Tables 1 and 2.

(b) The percentage of large mononuclears decreases up to the ninth week.

(h) *Tentative Diagnoses*

At the time I felt justified in diagnosing the cases of Table 2 as malaria: Table 3 now caused further speculation.

I began to suspect that the 30 men of this table had suffered from dengue fever for the following reasons :—

1. Dengue fever was extraordinarily prevalent in Bengal and the district in which these men had been. I did not know of this fact at the time the men were in hospital.
2. Having learnt of this epidemic, I paid closer attention to the charts of these 30 men, now disentangled from the charts of men in Table 2, and found that these charts in the majority of instances showed the characteristic rise of temperature of dengue on the last day of fever. The precise day of fever cannot be worked out, as these investigations were made at too late a date for the actual patients, now out of hospital and well, to remember the exact date on which they fell sick in the Tista Valley before admission to hospital.
3. On coming to classify the cases and tabulate their names, regimental numbers, etc., I was immediately struck by the fact that all the men of Table 3 were men from one company, and this company had been no further along the

valley than the first post. Further, no case of malaria occurred in this company, 28 of the cases falling to two other posts, while the twenty-ninth case was contracted whilst the patient was on furlough.

(i) *Subsequent behaviour substantiating diagnoses*

All these cases, followed further, are of interest.

Men of Table 1 were placed on a quinine roster, and were advised to attend hospital weekly on Saturdays and Sundays for 10 grain dosage. Practically none availed themselves—hence a number of relapses occurred, and a Battalion Order became necessary.

Men on Table 2 were not placed on the roster, partly in order to see whether the diagnosis was correct, which, in view of the fact that Anophelines were not present in this station, I felt would be more assured if relapses were to occur.

Men of Table 3 were not placed on any after-treatment.

In the course of time the following results were noted:—

1. Two men of Table 2 relapsed with malaria, and as two more at the time of the count showed pigment in the large mononuclears, we may be sure that 4 of the 10 cases diagnosed by count suffered from malaria at their primary attack.

All these cases did not relapse, probably for two reasons:—

- (a) Some had had a fairly severe dosage with quinine whilst in hospital. (Some, on the contrary, had none.)
- (b) As this was a hill station, we may suppose the effect of climate to be beneficial to recovery unaided by quinine, on the same principle that men with malaria, who cannot take the drug, are sent to the hills for the effect of such climate.

2. No man of Table 3 relapsed or came into hospital with any form of tropical fever during the ensuing eleven months. Beyond this, it is impossible to deduce anything further, as furlough or change of station supervened for these men, and so chances of malarial infection were re-obtained.

(j) Summary

Now, the corroborative evidence being strongly in favour of a correct diagnosis having been made with respect to 29 cases of malaria, and a correct 'exclusive' diagnosis of the remaining 30 (i.e. not malaria), I began to place considerable reliance upon the value of a differential blood count.

The following questions presented themselves for elucidation:—

1. What was the average count of healthy men of the battalion?
2. Was an increase of large mononuclears found in any other disease with which I had to deal?
3. Did such an increase occur at all constantly in malaria?
4. If an exclusive change in malaria, above what figure could one diagnose a case as malaria upon a count alone?
5. How long did the rise in large mononuclears remain during convalescence?
6. Could subsequent counts be made the scientific basis upon which after-treatment depends?

To arrive at the answers to these questions, I began by taking the blood of every man who came into hospital and of any others who could be persuaded to make the sacrifice in the interests of science.

V. SURVEY OF COUNTS IN HEALTH AND DISEASES OTHER THAN MALARIA

(a) Differential Count in Health

See Table 4. This table gives the counts made from 23 healthy men. These men were selected carefully, that is to say, at the time the blood was taken no disease was apparent or complained of, and later, if an examination of any man's medical history sheet disclosed an entry during the last two years of a disease likely to influence the count, e.g. malaria, syphilis, anaemia, etc., this count was discarded. There remained, of course, the element of chance that these men had suffered from some complaint during the furlough season, when no official check on their state of health can be kept.

A relative Eosinophilia will be noticed in certain cases. I had

no opportunity of investigating the question of worm infection. It is a fact, however, that worm infection is very prevalent amongst these Gurkha people, and also was much complained of amongst the neighbouring tea-garden coolies.

The principal fact to be noticed is that the arithmetic average of the large mononuclear leucocytes comes up to 4.68 per cent., a figure slightly higher than that found in text-books for European races.

Reference has been made in paragraphs 2(b) and 3(a) to an alteration in the standard of differentiation between the large and small lymphocytes. My first standard was to call any lymphocyte larger than the familiar shot-like small lymphocyte a large lymphocyte. An error, then, enters into this table, Table 3 and a few of the earlier malaria counts in which I adopted this first standard. As the malaria counts are much more numerous, I omit any correction for these earlier counts.

As some stress is laid later upon the normal percentage of large lymphocytes, a correction for this class of cell is now necessary in this table, for the percentage over the small lymphocyte group is too high, owing to the inclusion of lymphocytes smaller than the polymorphonuclear leucocyte.

The change in the standard of differentiation was decided upon in order that comparisons would be possible between my later counts and those of other observers who use the nomenclature advocated by Lieut.-Col. Sir L. Rogers, I.M.S.

This correction I take to be about 5 for the following reasons:—

1. Other observers, following Sir L. Rogers' nomenclature, which defines a large mononuclear as any mononuclear cell as large or larger than a polymorphonuclear (and therefore combines my large mononuclear and large lymphocyte), obtain an average for 'large mononuclears' of 10, whereas my average for true mononuclears comes to 4.68. The difference—5.32—should equal the percentage of large lymphocytes. This would give me a rather high correction figure of $12.58 - 5.32 = 7.26$.
2. By a rather abstruse method of calculation, which may be open to error, I obtain what may be termed a healthy average from the next three tables, i.e., Tables 5, 6 and 7. These tables deal with lobar pneumonia, broncho-

pneumonia, and bronchitis, and in each table a relative increase of polymorphonuclears is noticeable. It is fairly safe to infer that, in all these diseases, an actual increase of this first class of cell occurs and that the leucocytosis only affects this cell; were this not so, one would expect a very much greater discrepancy between the relative percentages of the other cells which are found after employing the method of 'reduction to normal' shown below.

Let us first take a simple case. Assume 10,000 cells to be the average number found in 1 cubic millimetre of healthy blood. Assume the differential count to be:—

Polymorphonuclear	61'00
Eosinophile	6'00
Mast cell	—
Large mononuclear	3'00
Large lymphocyte	9'00
Small lymphocyte	21'00

Then in 1 c.mm. of blood there are

Polymorphonuclear	6,100
Eosinophile	600
Mast cell	—
Large mononuclear	300
Large lymphocyte	900
Small lymphocyte	2,100
				10,000

Assume in a case of pneumonia that the leucocytosis reaches 30,000 in 1 c.mm. of blood and that the excess 20,000 cells are all polymorphonuclear.

Then the number of cells in 1 c.mm. of diseased blood =

Polymorphonuclear...	...	26,100, or a percentage of	87'00
Eosinophile	600, „ „	2'00
Mast cells	— „ „	—
Large mononuclear	...	300, „ „	1'00
Large lymphocytes	...	900, „ „	3'00
Small lymphocytes	...	2,100, „ „	7'00

It is now easy to see that, if we are given the last count, we can find a figure representing the actual increase, which, when deducted, will make the polymorphonuclears equal to the average found in health and at the same time restore the other cells to their normal average.

In applying this method to Tables 5, 6 and 7 it will be better to combine the averages of the polymorphonuclears and eosinophiles, owing to the fact that certain cases in the healthy table show marked eosinophilia at the expense of the polymorphonuclears.

The averages of these four tables, readjusted thus, are given below :—

	Polymorpho- nuclears and eosinophiles	Mast cells	Large mono- nuclears	Large lympho- cytes	Small lympho- cytes
Table 4—Health	67·64	0·42	4·68	12·58	14·67
„ 5—Pneumonia	88·13	0·11	1·93	3·01	6·82
„ 6—Broncho-pneumonia ...	75·37	0·11	2·15	5·56	16·81
„ 7—Bronchitis	73·44	0·13	2·68	5·93	17·82

If we assume the super-added increase in 1 c.mm. of blood to be 17,250, 3,140 and 2,180 in these diseases respectively, and employ the above method of reduction, we obtain new averages as below :—

	Polymorpho- nuclears and eosinophiles	Mast cells	Large mono- nuclears	Large lympho- cytes	Small lympho- cytes
Table 5—28 cases	67·65	0·29	5·25	8·20	18·58
„ 6—9 cases	67·63	0·14	2·82	7·50	22·08
„ 7—16 cases	67·64	0·15	3·26	7·22	21·70

The arithmetic average of these new counts, taking into account the number in each series, comes to :—

	Polymorpho- nuclears and eosinophiles	Mast cells	Large mono- nuclears	Large lympho- cytes	Small lympho- cytes
	67·64	0·23	4·24	7·787	20·12
as against	67·64	0·42	4·68	12·58	14·67

or a total of lymphocytes in health of 27·25 as contrasted with the new figure of 27·91.

The figure of correction, then, should be $12.58 - 7.79 = 4.79$. This would make the healthy averages as follows:—

Polymorpho-nuclears	Eosinophiles	Mast cells	Large mononuclears	Large lymphocytes	Small lymphocytes
56.63	11.01	0.42	4.68	7.79	19.46

(b) *Differential Counts in Lobar Pneumonia*

See Table 5. These slides were always interesting. A positive leucocytosis could at once be inferred from the superabundance of polymorphonuclear leucocytes seen in a given field. In the absence of a haemocytometer, the leucocytosis can be roughly inferred by the method of 'reduction to normal' shown previously.

The enormous increase of polymorphonuclears, the highest of the series being 95 per cent., suggested to me a pneumonic infection; but here I would mention that I never had to deal amongst these Gurkhas with other septicaemias, appendicitis, acute suppurations, etc.—on the contrary, the bulk of admissions were for pneumonia, bronchitis, malaria and other fevers, and minor injuries. Thus, remembering the incidence of lung diseases, I, in nine cases, diagnosed pneumonia by the microscope without having seen the patient, and a visit to the ward later in each instance confirmed the accuracy of the diagnosis.

I lay no stress on this reverse method of diagnosis: the counts, however, are of great value in corroborating a clinical diagnosis.

It will be noticed that the eosinophiles decrease both relatively and positively—a fact in accordance with the results of other observers. This decrease seems to vary directly with the increase in the polymorphonuclears.

(c) *Differential Counts in Broncho-Pneumonia*

See Table 6. The affection of broncho-pneumonia, being less severe than the last disease, calls for less increase in the polymorphonuclears.

(d) *Differential Counts in Bronchitis*

See Table 7. A drop in the average number of polymorphonuclears still occurs, whilst the large mononuclears are more approximating to the figure found in health.

(e) *Differential Counts in an Epidemic of unknown origin*

See Table 8. The seven counts of this table are certainly striking, but, unfortunately, no diagnosis can be given to the causal condition. The smears were sent to me by a native tea-garden doctor, who described the disease as being epidemic and very fatal. The few symptoms given to me were that the disease resembled pneumonia, but the condition relapsed and different areas of lung were affected.

At the same time there was a similar epidemic prevailing amongst the native population of Darjeeling, and in some of these cases a plague-like bacillus was isolated from the sputum. The bacillus was not that of plague, however, and no further diagnosis was arrived at. This fact would tend to negative the assumption that the disease was enteric fever—a supposition which the counts would support.

The lymphocytosis is so marked that the disease is probably a distinct entity.

(f) *Differential Counts in other diseases*

See Table 9. This table shows the results found in all the different diseases which passed through my hospital. One or two cases were specially examined when the diagnosis was certain, to see if a known change could be recorded.

Thus, the lymphocytosis of syphilis is shown in Case No. 26D. It will be noticed that this protozoan infection does not increase the percentage of large mononuclears according to my nomenclature, but does increase the large lymphocytes which others classify as large mononuclears.

Infective polymorphonuclear leucocytosis is shown in Case Nos. 18D and 25D.

Case No. 14D was interesting. This patient was a Sikh woman who suffered from abdominal pain, low pyrexia in the evenings, alternate diarrhoea and constipation, anorexia and gradual but persistent loss of weight. On examination I could make out little except a feeling of hard lymphatic glands in the mesentery. The diagnosis looked like *Tabes mesenterica*, but I suggested doing a differential blood count before giving an opinion. This showed 15 per cent. of eosinophiles. Now this percentage, compared with those found amongst Gurkhas, was not startling, but I had reason

to believe that up-country people possessed counts more approximating to those given in text-books. Therefore I looked upon this count as a possible indication of worm infection, and elected to try santonin.

A large number of *Ascaris lumbricoides* was passed under this treatment, and again after a subsequent dose, and during the ensuing month a complete recovery from all symptoms was effected.

Case No. 12D had a moderately high large mononuclear count. This man had had malaria some months previously, and complained of debility at the time of examination. The blood showed diffuse* basophilia, and I think it is almost certain, from his clinical symptoms and from the improvement effected by quinine and arsenic, that this count was raised by reason of past malaria.

Case Nos. 27D-38D cannot be given a more precise diagnosis than 'chill with fever.' None had any symptoms of malaria, all yielded to diaphoretics and only remained in hospital for two or three days.

(g) *Summary*

All the foregoing counts, with the one exception mentioned, were made from the blood of Gurkha people. Table 10 (a) shows the counts of 39 malaria cases taken from the same race and on the day of attack.

The averages of these tables are now tabulated below:—

	Cases	Counts	Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes	Broken nuclei
Health	23	24	56.63	11.01	0.42	4.68	12.58	14.67	—
Corrected average ...	—	—	56.63	11.01	0.42	4.68	7.79	19.46	—
Pneumonia	28	28	86.63	1.50	0.11	1.93	3.01	6.82	—
Broncho-pneumonia ...	9	9	71.48	3.89	0.11	2.15	5.56	16.81	—
Bronchitis	15	16	67.92	5.52	0.13	2.68	5.93	17.82	—
Epidemic, unknown origin	7	7	44.14	0.36	—	—	4.28	51.22	—
Other diseases, highest large mononuclear percentage	38	39	—	—	—	6.66	—	—	—
Malaria, benign tertian ...	28	28	60.05	4.73	0.32	14.56	3.52	16.64	0.18
„ malignant tertian	11	11	50.71	5.64	0.33	16.20	3.09	24.03	—

* *Diffuse Basophilia or Polychromasia.* Rarely did the preponderance of red corpuscles show this change. In scattered corpuscles it was common, and then almost invariably in malarial bloods.

It is evident that an appreciable increase of large mononuclears occurred in no disease with which I had to deal, other than malaria. Furthermore, this increase in malaria was almost constantly found.

At this period of the investigations I came to look upon a large mononuclear count of from 8 to 10 per cent. as suspicious of convalescence from malaria, and one of 10 per cent. or over as diagnostic of 'latent' or actual infection. The meaning which I assign to 'latent' malaria will be exemplified later. I consider it safer to place the figure upon which to diagnose malaria at a percentage at least double that found as an average in normal bloods, in view of the many errors which may enter into such counts.

VI. OBSERVATIONS UPON BLOOD DURING CONVALESCENCE FROM MALARIA

(a) *Method employed*

In order to find out how long, during convalescence, the large mononuclear count remained above 10 per cent., I carried out a rather disconnected series of counts on patients of Table 10 (a). Many more would, doubtless, have been made, had these men been in hospital instead of on duty.

Each count is entered in Table 11 as 'so many days after the first attack.' Provided no relapses occurred, averages could be struck immediately for the groups of counts in each week, but, as presumably each relapse will again influence the output of large mononuclears, it is necessary to re-group the counts (as in Table 12), and to treat each relapse as a fresh attack. Subsequent counts are then arranged at their proper interval from the last relapse. For the sake of clearness, all cells other than the large mononuclears are excluded from Table 12.

(b) *Results of tabulation*

By this method of bringing counts of relapses back to the 'first day of fever,' we note that the average large mononuclears increase from 15.02 per cent. (the average of all malarias, first day, amongst Gurkhas) to 16.88 per cent., this rise doubtless being due to the fact that severer infections are being dealt with.

As would be expected, the large mononuclear average declines from its height on the first day until it reaches a normal anywhere

from the twelfth week onwards. (One case, Case No. 19, is peculiar as the large mononuclear average remained low during the fever, and rose during convalescence.)

Up till the eighth week the average would appear to remain at or above 10 per cent. This point I lay great stress on, although the exact period of time may come to be altered with more observations.

This discovery has repeatedly been useful to me in practice. I have been consulted as to the probable diagnosis of fever contracted whilst shooting in the jungle, and recovered from by the time a doctor could be consulted. In such a case, if I find a high large mononuclear count, I unhesitatingly say that the disease was malaria, and advise a course of quinine treatment.

From the nineteenth week onwards, there is a larger group of counts, some of which were made whilst a new complaint existed. The fact that no malaria relapses occurred, when the resistance may be said to have been lowered by reason of a fresh illness, would go to prove that, with the large mononuclear count reduced to normal, 'latent' malaria was completely eradicated.

(c) *Use of Count in treatment*

Both Table 12 and the Table next to be discussed (Table 13) show that cases with high large mononuclear counts tend to relapse. Table 12 also shows that the normal average is not reached under quinine treatment until the twelfth week of convalescence; from this it would follow that after-treatment should be continued for at least three months.

Sir Ronald Ross lays down four months as a safe margin for the entire eradication of this disease; these figures support that contention.

My own practice in this battalion was to keep each malarial patient in hospital for one month, and to administer thirty grains of quinine per diem. After one month, if fit to be discharged, the case returned to duty and attended hospital twice weekly for ten grains of quinine until two more months had passed. In later cases I made use of the differential count to determine whether quinine should be discontinued. Only those men remained on the roster whose large mononuclear averages continued high.

VII. USE AND ACCURACY OF DIAGNOSING MALARIA BY COUNT ALONE

(a) *In Non-malarious Station*

The importance of this station being non-malarious must again be emphasised, as upon this fact depends the value of the ensuing investigations. The principle is simple, and consisted only in waiting for relapses in men whom I was led to suspect were malarially infected. The expectation was that these men, diagnosed solely upon their large mononuclear count, would relapse in at least as high a proportion as the known malarial cases relapsed.

See Table 13. In all, 22 men were watched. In 11 no further symptoms were noticed. The histories of the remaining 11 are now detailed.

4 men, mentioned in Table 2, are already known to be correctly diagnosed.

1 man, Case No. 34, returned from furlough, complained of debility, looked anaemic, showed a count of 19 per cent. large mononuclears, and was (perhaps rather unfairly) left without quinine treatment. Two hundred days later, during which interval the man had never left this non-malarious place, he returned with an attack of benign tertian malaria.

1 man, Case No. 35, relapsed in a shorter interval of thirty-two days.

1 man, Case No. 105, had his blood taken as a routine measure without any disease being complained of. The blood showed no parasites or any change indicative of malaria except a high large mononuclear percentage. The next day he was admitted to hospital with benign tertian malaria.

4 men remain to be discussed, and provide the best example.

Recruits, arriving from badly infected districts of Nepal, provided an excellent field for research. Not only did they frequently relapse with malaria, as experience had taught, but such relapses followed quickly upon their being made to undergo physical training.

I obtained the blood of 16 recruits at the time of the first

vaccination. Of these, 6 showed a large mononuclear count of over 10 per cent., and so were noted. These first counts are unfortunately missing. Of these 6, 4 came into hospital within the following three months with attacks of benign tertian malaria. (See Table 10 (a), Serial Nos. 10-12 and 20.)

Of the 10 recruits showing low counts, none were admitted at all.

Thus, out of 22 cases diagnosed upon count alone, 11 cases ultimately relapsed—surely rather a high percentage if the result were pure chance.

The cases of known malaria followed in Table 12 relapsed, despite quinine treatment, in the proportion of 1 to 3 (or 25 per cent.). The fact that 50 per cent. of the above cases relapsed, while doubly in favour of the correctness of my theory, is explainable probably by the fact that none of these suspected cases had undergone any treatment.

The reverse side of the picture must also be taken into account. There are 52 cases where malaria was excluded on count: these are 30 'dengue' cases, the 10 recruits mentioned, and 12 cases diagnosed only as 'chill with fever.' Out of these, none betrayed malaria by admission up to the time when the battalion left this non-malarious station—a period varying with each case from one year to one or two months.

(b) *In a malarious station*

In November, 1913, the battalion left for the plains and became split up into detachments.

From one detachment I kept receiving, monthly, very large admission rates, principally for malaria. Thus in a little over three months there were 218 admissions for 114 rank and file, of which 182 were for malaria. These 182 admissions were for 69 men.

As the season was unfavourable to such an epidemic, and the number of days spent in hospital by many cases was very small, I began to doubt the accuracy of the diagnoses. I decided to visit the district and, besides surveying the sanitary state of the camp, etc., to employ this method of blood-counting to estimate as far as possible the gross malarial infection.

It will be seen that I accepted the blood count method as accurate, and was about to use it as a standard. The probability was that this method had more to commend it than the rapid

diagnosis by thermometer and pulse-rate, which I found was practically all that was employed on the spot.

I was able to obtain the blood from 56 of these 69 men, and also from some who had had so far no admissions for sickness; 15 men were actually in hospital at the time of my visit, and 2 more who had had no previous illness were acting as sick attendants.

In the case of these 17 men, a clinical examination was possible. I refrained, however, from making this at once, in order that the examination might later be made use of as a check on the accuracy of my diagnoses arrived at from blood-counting.

First, I drew out a chart somewhat similar to Table 14 (q.v.), on which each of the 56 men was entered, along with the diagnosis returned, against the date of his last admission. The dates were arranged so that the last date came at the top. This date was March 5th, 1914, and on the same date all the blood smears were taken. We have already seen that in convalescence from malaria, the large mononuclear percentage remains at or above 10 per cent. for eight weeks. Therefore, eight weeks distant from March 5th a red line was drawn across the chart. Above this line, any case showing a large mononuclear count of 10 per cent. or higher could justifiably be diagnosed as having had malaria; below this line a doubt would remain if the count were lower.

With the 17 men capable of being examined in hospital, the method was to take blood-smears and to number the slides only with their regimental numbers—figures which one does not remember in association with a man's name. These counts were then made, but were not copied on to the chart.

The following day I examined each man under his own name, came to a diagnosis where possible, and then entered this diagnosis in the appropriate column of the chart. When these entries had been made (and not till then), the blood findings were entered from another book.

The results are, I think, strikingly interesting.

13 men were formerly diagnosed as malaria; of these, in

5 I clinically diagnosed malaria, found parasites and counts above 10 per cent.;

1 I clinically diagnosed malaria, found pigment and a count above 10 per cent.;

- 2 I clinically diagnosed malaria, and found counts above 10 per cent.;
- 3 I found no appreciable disease, and no evidence of malaria from charts, spleen, etc., and the large mononuclear count was low; and in
- 2 I found slight leucocytosis with polymorphonuclear increase, and clinically found lymphangitis and adenitis, although malaria was the return.

Four men were left, one with an old gumboil, one with dyspepsia, and two were the sick attendants. In all four of these I found low large mononuclear counts and a diagnosis compatible with that returned.

In these cases I could be sure of the accuracy of the findings.

With the remainder of the men, as I could make no examination, I could only scan their charts and enter remarks as to these on my chart. Here, the counts, in the light of the previous blood work, would warrant the assertion that diagnosis was wrong in 10 cases out of 24, and previous to January 8th was correct in 7 cases out of 15, after omitting the cases which showed parasites, and so were relapsing at the time of count. In these very old cases, the diagnosis was probably correct in 3 more, i.e., those giving large mononuclear percentages of 9.00, 9.50, and 9.33, respectively.

(c) *Further proof of theory*

In May, 1914, I left this Gurkha battalion and took up a brigade laboratory appointment in the Punjab.

The races of people now dealt with were mainly Jats, Sikhs, Dogras, and Punjabis. Forty-four more counts were made in malaria at the time of attack, and these are given in Table 10 (b). It is noteworthy, *en passant*, that the average of the large mononuclears in 42 benign tertian infections closely approximates to that found for 28 similar cases amongst Gurkhas, i.e., 14.46 per cent. : 14.56 per cent.

In the case of Serial Nos. 72-81 of Table 10 (b) and 261-267 of Table 13, examination was first made for parasites, the time allowed being, roughly, five to ten minutes in each case. No parasites were seen. Next, a lengthy blood count of 400 cells was made with each slide, and, the attention being perhaps too much directed to the white cells, no parasites were seen.

In each of these 17 counts a high percentage of large mononuclears was recorded.

In all previous counts of a similar nature, when parasites were not seen, the investigation stopped here, and the diagnosis was entered as 'diagnosed as malaria on blood count.'

But, as Sir R. Röss points out, the chance of finding a parasite depends directly upon the time expended. The total volume of blood in a man of average weight, he estimates, will contain 15,000,000,000,000 red blood corpuscles, and fever will not supervene in an infected man until there are 150,000,000 parasites.

Thus, in an early infection, 100,000 red blood corpuscles may have to be examined before a parasite is seen. Chance will, of course, make this number greater or less. If we assume an average of 200 corpuscles to one field, and that 20 fields are examined per minute with a one-twelfth oil immersion objective, it may be twenty-five minutes before a parasite is discovered.

Feeling convinced that the diagnosis by count was correct in these 17 cases, and remembering the factor of time in finding a parasite, I decided to continue the search. In 10 cases I was ultimately rewarded. Only one fever form was seen in each smear, and the shortest time spent in this continued search was thirty minutes; in the longest case, sixty-five minutes.

In two more cases pigment was seen, and finally the diagnosis of one case, Serial No. 266, Table 13, was confirmed later, the medical officer in charge notifying me that the patient had a sharp attack of benign tertian twenty-three days previously.

(d) *Summary*

The differential blood count has been the means whereby a diagnosis for or against malaria was arrived at in 103 cases.

42 cases were diagnosed as malaria by counts of 10 per cent. or over; 61 cases were diagnosed as not malaria by low counts.

Of the 42 cases, 27 were correctly diagnosed.

Of the 61 cases, 6 were definitely not malarial, 52 were followed for some months and did not relapse, and in 3 no further decision could be made.

(e) *'Latency' of malaria*

This work leads to the question: How does malaria remain 'latent'? Do we believe in encystment, in parthenogenesis, or simply

in diminished numbers? Personally, I incline to the last view, and if this is accepted, we can easily see how a total infection of (say) 150,000 parasites would require weeks of constant search, day and night, for the discovery of one parasite.

The presence of diminished numbers of parasites free in the blood-stream would reasonably continue to exert a toxic effect, and, if the toxic irritation is the factor in producing a large mononuclear increase, this increase will be maintained.

Whereas I looked upon a high large mononuclear count, at first, as indicative of a *past* infection, now I view it as a sign of either a very early *actual* infection with parasites or a diminishing infection. The blood is quick to react to purulent infection, and the leucocytosis again quickly recedes when the infective matter is removed. The mere abolition of fever, in malaria, does not, however, remove the infection.

Thus it appears to me that the method of diagnosis by blood count not only acts as an incentive to further search for parasites in early or late cases, but *also*, in the event of failure to find these, constitutes a fairly accurate method of diagnosis.

VIII. GENERAL OBSERVATIONS

(a) *Origin of broken nuclei*

It has already been remarked that broken nuclei were more abundant in malarial bloods. They therefore have some pathognomonic significance.

As to their origin, it is plain that neither the polymorphonuclear, the eosinophile, nor the mast cell can give rise to them, as these cells, when ruptured in a smear, betray themselves by their closely-scattered granules.

Again, the variation in the number of broken nuclei according to the method of slide-preparation, puts out of court any supposition that they may originate from other body-cells which are not present in drawn blood.

The small lymphocyte can also fairly be excluded, as this cell is relatively abundant in healthy bloods where broken nuclei are rarely seen. Since the time when I began to count the broken nuclei separately, there are many counts which show a very high percentage of small lymphocytes, and yet no broken nuclei are noted.

Between the large lymphocyte and the large mononuclear leucocyte the decision must therefore be made. My reasons for thinking the nuclei originate from the latter are three:

1. The impression left after many counts is that they were more numerous in malarial bloods, in which the other noticeable change is one of increase in the large mononuclears and decrease in the large lymphocytes.
2. The broken nuclei stain with a similar shade of colour to the nuclei of whole large mononuclears.
3. A mathematical reason. Studying the only (apparently) normal bloods in which full classification has been adopted, i.e., in Table 14, we find 23 cases in which 4,250 cells were counted, and 10 broken nuclei were noted. Against this there are 53 malarial counts in which 19,250 cells were observed and 128 broken nuclei were seen.

Thus:

4,250 healthy cells gave 10 = 0.002109

19,250 malaria „ „ 128 = 0.006649

The proportion is 2109 : 6649 = 1 : 3.15

= 4.68 : 14.74

or the same proportion as the healthy large mononuclear average is to the large mononuclear average in all malarial counts (i.e., 14.78).

The extraordinary nearness of these figures should not mislead, as the observations are few. I would prefer to lay more stress on the first two reasons.

It sometimes happens that a duplicate cannot be obtained of a slide in which broken nuclei figure to excess. In such cases it is worth considering whether the nuclei cannot be enumerated as large mononuclears. If the number is excessive, and still more if the irregular smearing, so peculiar to malarial bloods, and a slight degree of basophilia, punctate or diffuse, are present, then I should feel justified in suggesting the case was malaria.

(b) *Plasmodium tenue*

The last count in Table 10 (b) was made from a blood containing numerous parasites, exactly similar to the peculiar forms described by Professor J. W. W. Stephens, and so named by him. As some

consider that it is an actively amoeboid form of the malignant tertian parasite, I mention, for what it is worth, that this would constitute the only malignant parasite seen by me in a station where malaria was very prevalent.

(c) *The advantages of the nomenclature used*

There are many ways of using these Tables for making comparisons with the results obtained by adopting a different classification for the large mononuclear cell.

It is possible to reconstruct Tables 4 and 10 (*a, b*) so that each count and the final average will be in conformity with the nomenclature advocated by Lieut.-Col. Sir L. Rogers, I.M.S.

This result will be achieved if the large mononuclear and large lymphocyte percentages are added together; in Table 4, however, as a correction has been made for the average of the whole group, each separate figure for large lymphocytes must also be corrected. This involves much reckoning, all of which is omitted here.

Having arrived at these new results, we can then compare the two sets of tables by applying the test as to how many cases in health and malaria will be incorrectly diagnosed by accepting certain standards for the determination of malarial infection.

The average count for 83 cases of malaria is :

Polymorpho-nuclears	Eosinophiles	Mast cells	Large mononuclears	Large lymphocytes	Small lymphocytes	Broken nuclei
58.61	2.99	0.24	14.79	2.64	20.31	0.41

The new 'large mononuclear' percentage will therefore be $14.79 + 2.64$, which equals 17.43.

The standard adopted must be below this figure; as it is a purely arbitrary one, different errors will result if we alter it. This being so, let us try the effect of different standards, using 13, 14, 15 and 16.

A series of counts in health and malaria is published by Captain H. Stott, I.M.S., in the *Indian Medical Gazette*, March, 1915, and as these are based on the nomenclature under discussion, the same comparison can be made with these. (It must be noted that my tables and his are not strictly comparable, as he omits mast cells, broken nuclei, and decimal fractions in his counts, and so introduces an error throughout.)

The following approximate percentages of cases will be found to be incorrectly diagnosed, i.e., certain healthy counts will be adjudged malarious and certain malarial bloods will be accounted healthy :

Standard of		13	14	15	16
Tables 4 and 10 (a, b) :—		per cent.	per cent.	per cent.	per cent.
Health (23 cases); diagnosed as malaria	26	26	26	26
Malaria (83 cases); diagnosed as healthy	17	27	35	44
Captain Stott's counts :—					
Health (25 cases); diagnosed as malaria	16	16	12	8
Malaria (163 cases); diagnosed as healthy	22	26	30	39

A simple way of writing these errors is to assume that 50 healthy bloods and 50 malarial bloods are examined. When the 100 counts are then studied with these standards, the percentage errors will be :

		13	14	15	16
		per cent.	per cent.	per cent.	per cent.
My Tables	21.5	26.5	30.5	35.0
Captain Stott's Tables	19.0	21.0	21.0	23.5

Now let us examine the percentage errors obtained when the large mononuclears are disentangled from the large lymphocytes, again using different standards.

Standard of		8	9	10	11	12	13
Tables 4 and 10 (a, b) :—		per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Health (23 cases); diagnosed as malaria	17.0	8.5	—	—	—	—
Malaria (83 cases); diagnosed as healthy	1.2	1.2	3.6	10.8	19.0	36.0
Or with 50 healthy bloods and 50 malarial bloods, error will be	9.1	4.8	1.8	5.4	9.6	18.0

Comparing the most favourable standards above, we find the error is very much reduced by adopting this fuller nomenclature. It is also obvious that the best results are obtained by using a standard of 10 per cent. large mononuclears as indicative of malarial infection—in fact, I did so adopt it for precisely these reasons.

TABLE 4

DIFFERENTIAL BLOOD COUNTS IN HEALTH

Serial No.	Case No.		Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
84	1H	69.00	8.00	—	4.00	4.00	15.00
85	2H	61.00	13.00	—	4.00	9.00	13.00
86	3H	64.00	22.00	—	3.00	9.00	2.00
87	4H	65.00	13.00	1.00	1.00	14.00	6.00
88	5H	55.00	4.00	1.00	8.00	15.00	17.00
89	6H	75.00	—	—	2.00	10.00	13.00
90	7H	59.00	7.00	—	7.00	8.00	19.00
91	8H	55.00	8.00	—	3.00	13.00	21.00
92	9H	62.00	8.00	—	7.00	6.00	17.00
93	10H	45.00	6.00	1.00	4.00	20.00	24.00
94	11H	55.00	17.00	1.00	1.00	2.00	24.00
95	12H	Before meals...	37.50	19.00	0.50	6.00	25.50	11.50
96	—	After meals ...	46.66	12.33	0.66	5.33	25.33	9.66
97	13H	61.00	7.00	1.00	9.00	3.00	19.00
98	14H	62.00	5.00	—	—	8.00	25.00
99	15H	63.00	7.00	—	5.00	8.00	17.00
100	16H	53.00	25.00	—	4.00	14.00	4.00
101	17H	Contusion ...	64.00	—	—	7.00	19.00	10.00
102	18H	„ ...	57.00	20.00	1.00	4.00	14.00	4.00
103	19H	Abrasions ...	37.00	15.00	1.00	9.00	25.00	13.00
104	20H	„ ...	48.00	11.00	2.00	5.00	26.00	8.00
105	21H	Sprain ...	53.00	17.00	—	2.00	7.00	21.00
106	22H	„ ...	47.00	17.00	—	4.00	9.00	23.00
107	23H	65.00	3.00	—	8.00	8.00	16.00
		Arithmetic average ...	56.63	11.01	0.42	4.68	12.58	14.67
		Corrected average (see Text)	56.63	11.01	0.42	4.68	7.79	19.46

TABLE 5

DIFFERENTIAL BLOOD COUNT IN LOBAR PNEUMONIA

Serial No.	Case No.							Poly-morpho-nuclears	Eosino-philes	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
108	1LP	95.00	—	—	3.00	—	2.00
109	2LP	93.00	—	—	4.00	1.00	2.00
110	3LP	92.00	—	—	3.00	4.00	1.00
111	4LP	92.00	—	—	3.00	2.00	3.00
112	5LP	87.00	1.00	1.00	1.00	3.00	7.00
113	6LP	80.00	—	—	5.00	5.00	10.00
114	7LP	81.00	2.00	—	2.00	5.00	10.00
115	8LP	79.00	2.00	—	3.00	4.00	12.00
116	9LP	84.00	5.00	—	1.00	3.00	7.00
117	10LP	95.00	1.00	—	—	1.00	3.00
118	11LP	+ Pleurisy c. Effusion					...	95.00	—	—	1.00	3.00	1.00
119	12LP	80.00	1.00	—	—	7.00	12.00
120	13LP	91.00	—	—	1.00	4.00	4.00
121	14LP	76.00	10.00	—	2.00	3.00	9.00
122	15LP	80.00	1.00	—	1.00	5.00	13.00
123	16LP	94.00	—	—	—	2.00	4.00
124	17LP	89.00	—	—	3.00	2.00	6.00
125	18LP	87.00	2.00	—	3.00	1.00	7.00
126	19LP	93.00	—	—	—	4.00	3.00
127	20LP	90.00	—	—	1.00	1.00	8.00
128	21LP	90.00	—	—	—	3.00	7.00
129	22LP	79.00	7.00	1.00	1.00	5.00	7.00
130	23LP	91.00	2.00	—	—	1.00	6.00
131	24LP	84.00	8.00	1.00	2.00	1.00	4.00
132	25LP	75.00	—	—	4.00	9.00	12.00
133	26LP	85.00	—	—	4.00	—	11.00
134	27LP	86.66	—	—	—	3.33	10.00
135	28LP	82.00	—	—	6.00	2.00	10.00
		Arithmetic average	86.63	1.50	0.11	1.93	3.01	6.82

TABLE 6

DIFFERENTIAL BLOOD COUNTS IN BRONCHO-PNEUMONIA

Serial No.	Case No.						Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
136	1BP	77.00	1.00	1.00	2.00	2.00	17.00
137	2BP	69.00	5.00	—	1.00	5.00	20.00
138	3BP	83.00	—	—	3.00	6.00	8.00
139	4BP	83.33	2.00	—	3.33	2.00	9.33
140	5BP	61.00	1.00	—	1.00	15.00	22.00
141	6BP	66.00	11.00	—	3.00	—	20.00
142	7BP	65.00	1.00	—	2.00	9.00	23.00
143	8BP	76.00	—	—	1.00	7.00	16.00
144	9BP	63.00	14.00	—	3.00	4.00	16.00
		Arithmetic average	71.48	3.89	0.11	2.15	5.56	16.81

TABLE 7

DIFFERENTIAL BLOOD COUNTS IN BRONCHITIS

Serial No.	Case No.		Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
145	1B	47.00	20.00	1.00	6.00	19.00	7.00
146	2B	74.00	3.00	—	3.00	6.00	14.00
147	3B	65.00	1.00	—	3.00	9.00	22.00
148	4B	69.00	3.00	—	3.00	4.00	21.00
149	5B	76.00	2.00	—	5.00	3.00	14.00
150	6B	52.66	13.33	—	1.33	4.66	28.00
151	7B	55.00	6.00	—	1.00	10.00	28.00
152	8B	74.00	2.00	—	2.00	7.00	15.00
153	9B	77.50	0.50	—	6.00	8.50	7.50
154	10B	78.00	1.00	—	—	4.00	17.00
155	11B	85.57	2.57	—	0.57	1.71	9.57
156	12B	68.00	8.00	1.00	1.00	5.00	17.00
157	13B	60.00	12.00	—	3.00	3.00	22.00
158	—	5 days later	63.00	12.00	—	3.00	1.00	21.00
159	14B	69.00	1.00	—	2.00	6.00	22.00
160	15B	73.10	1.00	—	3.00	3.00	20.00
		Arithmetic average	67.92	5.52	0.13	2.68	5.93	17.82

TABLE 8

DIFFERENTIAL BLOOD COUNTS IN EPIDEMIC RELAPSING 'PNEUMONIA'

Serial No.	Case No.		Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
161	1ER 'P'	40.00	1.00	—	—	8.00	51.00
162	2ER 'P'	42.00	—	—	—	4.00	54.00
163	3ER 'P'	60.00	0.50	—	—	7.00	32.00
164	4ER 'P'	37.00	—	—	—	3.00	60.00
165	5ER 'P'	46.00	1.00	—	—	4.00	49.00
166	6ER 'P'	B.	38.00	—	—	—	4.00	58.00
167	7ER 'P'	46.00	—	—	—	—	54.00
		Arithmetic average	44.14	0.36	—	—	4.28	51.22

N.B.—No broken nuclei were seen in the above seven slides.

TABLE 9

DIFFERENTIAL BLOOD COUNTS IN OTHER DISEASES

Serial No.	Case No.		Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
198	1D	Active Pulm. Tuberculosis ...	49.50	10.75	1.00	5.75	—	33.00
199	2D	" " " ...	57.00	5.00	—	3.00	11.00	24.00
200	3D	" " " ...	68.00	9.00	—	3.00	8.00	12.00
201	4D	" " c. dry Pleurisy ...	77.00	4.00	—	1.00	7.00	11.00
202	5D	" " " " ...	63.00	8.00	—	3.00	6.00	20.00
203	6D	" " " " ...	81.00	—	—	2.00	7.00	10.00
204	7D	" " c. Pl. Effusion ...	57.00	4.00	—	3.00	10.00	26.00
205	8D	Acute Tb. Pneumonia ...	89.66	0.66	—	1.66	3.33	4.66
206	9D	" " " ...	79.00	3.00	—	4.00	4.00	10.00
207	10D	Anaemia and Debility ...	41.00	11.00	—	2.00	10.00	36.00
208	11D	" " " ...	71.00	1.00	—	2.00	2.00	24.00
209	12D	" Post-malarial ...	70.50	8.50	—	8.50	3.50	9.00
210	13D	<i>Ascaris lumbricoides</i> ...	59.33	19.33	—	—	0.66	20.66
211	14D	" " ...	59.00	15.00	—	2.00	4.00	20.00
212	15D	Dysentery ...	70.00	3.00	—	1.00	2.00	24.00
213	16D	Dyspepsia ...	39.00	7.00	—	6.00	22.00	26.00
214	17D	Enteric Fever, 1st day ...	80.00	3.00	1.00	1.00	5.00	10.00
215	—	" " 2nd day ...	79.00	1.00	—	—	9.00	11.00
216	18D	Gonorrhoeal Rheumatism ...	74.00	—	—	4.00	13.00	9.00
217	19D	Hepatitis. M. Bp. ...	79.00	—	—	2.00	10.00	9.00
218	20D	Myalgia Rheumatica ...	60.00	12.00	1.00	4.00	4.00	19.00
219	20D	" " later ...	58.00	13.00	—	1.00	1.00	27.00
220	21D	Orchitis ...	61.00	10.00	—	4.00	21.00	4.00
221	22D	Relapsing Fever (<i>Sp. carteri</i> ?) ...	57.00	1.00	—	2.50	12.00	27.50
222	23D	" " " ...	69.33	4.66	—	2.66	7.33	16.00
223	24D	Scabies ...	52.00	10.00	—	3.00	23.00	12.00
224	25D	Suppurative Mastitis ...	76.80	12.00	0.80	3.20	0.40	6.80
225	26D	Tertiary Ulcer ...	38.66	9.33	0.66	4.00	24.00	26.66
226	27D	'Chill with Fever' ...	86.00	7.50	—	1.00	—	5.50
227	28D	" " " ...	69.00	5.00	1.00	1.00	5.00	19.00
228	29D	" " " ...	51.00	16.00	—	—	2.00	31.00
229	30D	" " " ...	66.50	6.50	0.50	3.50	3.00	22.00
230	31D	" " " ...	67.33	4.66	—	1.33	6.00	20.66
231	32D	" " " ...	52.00	1.00	—	2.00	10.00	35.00
232	33D	" " " ...	58.00	13.33	—	6.66	1.33	20.66
233	34D	" " " ...	70.00	2.00	—	4.00	9.00	15.00
234	35D	" " " ...	78.00	8.00	1.00	1.00	3.00	12.00
235	36D	" " " ...	65.00	8.00	—	4.00	6.00	17.00
236	37D	" " " ...	68.50	10.00	—	0.50	1.50	19.50
237	38D	" " " ...	62.00	—	—	3.00	13.00	22.00

M. = Megalocytes.

Bp. = *Basophilus punctata*.

TABLE 10

DIFFERENTIAL BLOOD COUNTS IN MALARIA

(a) Amongst Gurkhas

Serial No.	Case No.	Remarks	Cells counted	Poly-morpho-nuclears	Eosino-philic	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes	Broken nuclei
1	1	BT. FF. ...	—	66.00	7.00	0.66	14.66	—	11.66	—
2	2	BT. FF. SF. ...	—	66.00	—	1.00	10.00	—	23.00	—
3	3	BT. FF. ...	—	55.00	12.00	—	14.00	4.00	15.00	—
4	4	BT. FF. P. ...	—	57.00	1.00	—	26.00	10.00	6.00	—
5	5	BT. FF. B. ...	—	65.00	—	—	18.00	12.00	5.00	—
6	6	BT. FF. SF. ...	—	78.00	2.00	—	15.00	4.00	1.00	—
7	30	BT. FF. ...	—	49.50	6.50	0.50	15.00	9.00	19.50	—
8	34	BT. FF. SF. ...	—	58.50	10.00	0.50	16.50	1.00	13.50	—
9	38	BT. FF. SF. ...	—	65.00	8.00	1.50	6.00	—	19.50	—
10	40	BT. SF. ...	—	74.00	6.00	—	10.00	2.00	8.00	—
11	41	BT. FF. SF. ...	—	75.00	3.00	—	12.00	3.00	7.00	—
12	42	BT. FF. Bp. ...	—	65.00	1.00	2.00	17.00	3.00	12.00	—
13	43	BT. FF. P. ...	—	36.33	26.66	0.33	19.00	3.00	14.66	—
14	44	BT. FF. B. Bp. P. ...	—	52.00	0.66	—	13.33	1.33	32.66	—
15	45	BT. FF. SF. ...	—	63.00	—	—	15.00	2.00	20.00	—
16	46	BT. FF. B. Bp. ...	—	55.00	2.00	1.00	14.00	3.00	25.00	—
17	47	BT. FF. SF. ...	—	52.00	2.00	—	18.00	14.00	14.00	—
18	48	BT. FF. SF. ...	—	71.00	4.00	—	18.00	2.00	5.00	—
19	49	BT. FF. SF. ...	—	58.00	8.00	—	11.00	2.00	21.00	—
20	51	BT. FF. ...	—	67.00	3.00	—	18.00	7.00	5.00	—
21	7	MT. FF. SF. B. ...	—	28.50	2.00	1.00	26.50	—	42.00	—
22	8	MT. FF. ...	—	37.00	19.00	1.00	8.00	2.00	33.00	—
23	9	MT. FF. SF. Bp. ...	—	49.00	2.00	—	22.00	5.00	22.00	—
24	10	MT. FF. ...	—	53.00	2.00	—	19.00	6.00	20.00	—
25	29	MT. FF. SF. ...	—	46.00	12.00	—	24.00	8.00	10.00	—
26	31	MT. FF. SF. B. Bp. N. ...	—	48.66	1.66	—	10.00	7.33	32.33	—
27	35	MT. FF. ...	—	54.00	3.00	1.00	18.00	2.00	22.00	—
28	36	MT. FF. B. P. ...	—	48.66	15.33	0.66	12.66	2.66	20.00	—
29	37	MT. FF. ...	—	71.00	—	—	17.00	—	12.00	—
30	50	MT. FF. SF. ...	—	80.00	1.00	—	10.00	—	9.00	—
31	52	BT. FF. B. ...	200	74.50	—	0.50	12.00	3.00	8.50	1.50
32	53	BT. FF. ...	100	65.00	4.00	—	12.00	4.00	13.00	2.00
33	54	BT. FF. SF. P. ...	150	42.00	2.00	—	12.66	3.33	39.33	0.66
34	55	BT. FF. P. ...	200	64.00	5.00	—	11.50	1.50	17.50	0.50
35	56	BT. FF. SF. ...	100	60.00	1.00	—	8.00	—	31.00	—
36	57	BT. FF. SF. P. ...	200	59.00	3.00	—	18.50	0.50	18.50	0.50
37	58	BT. FF. SF. ...	200	54.00	7.00	1.00	15.00	—	23.00	—
38	59	BT. FF. SF. P. ...	200	34.50	7.50	—	17.50	4.00	36.50	—
39	60	MT. SF. B. ...	100	42.00	4.00	—	11.00	1.00	42.00	—
Arithmetic average, Benign Tertian, Serial Nos. 1-20; 31-38 ...			—	60.05	4.73	0.32	14.56	3.52	16.64	0.18
Arithmetic average, Malignant Tertian, Serial Nos. 21-30 and 60 ...			—	50.71	5.64	0.33	16.20	3.09	24.03	—

ABBREVIATIONS

BT. = Benign Tertian
 MT. = Malignant Tertian
 FF. = Fever forms
 SF. = Sexual forms

B. = *Basophilia diffusa* or Polychromasia
 Bp. = *Basophilia punctata*
 P. = Pigment in Large Mononuclears
 N. = Nucleated Red Cells seen

TABLE 10.—(Continued)

(b) Amongst Sikhs, Jats, Dogras and Punjabis

Serial No.	Case No.	Remarks	Cells counted	Poly-morpho-nuclears	Eosino-philcs	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes	Broken nuclei
40	61	BT. FF. ...	400	72.00	—	—	14.00	—	14.00	—
41	62	BT. FF. ...	400	67.00	1.00	—	12.00	3.00	15.50	1.50
42	63	BT. FF. Bp. ...	400	66.00	—	1.00	13.00	7.00	12.00	1.00
43	64	BT. FF. ...	400	74.00	1.00	—	13.00	—	11.00	1.00
44	65	BT. FF. ...	400	67.00	1.00	—	13.00	3.00	16.00	—
45	66	BT. FF. SF. B. ...	400	63.00	—	—	12.00	—	25.00	—
46	67	BT. FF. SF. B. ...	400	42.00	9.00	—	19.00	3.00	25.00	2.00
47	68	BT. FF. ...	600	37.33	1.33	0.66	14.66	4.00	41.00	1.00
48	69	BT. FF. SF. ...	400	79.00	—	—	10.50	1.00	9.50	—
49	70	BT. FF. SF. ...	400	60.00	1.00	—	15.00	3.00	20.00	1.00
50	71	BT. FF. ...	400	75.00	1.00	—	15.00	1.00	6.00	2.00
51	72	BT. FF. SF. ...	400	45.00	—	—	15.00	5.00	34.00	1.00
52	73	BT. FF. SF. B. ...	400	65.00	1.00	—	14.00	—	20.00	—
53	74	BT. FF. P. ...	400	72.00	—	—	12.00	1.00	15.00	—
54	75	BT. FF. P. ...	400	64.00	—	—	14.00	2.00	18.00	2.00
55	76	BT. FF. P. ...	400	41.00	1.00	—	19.00	6.00	33.00	—
56	77	BT. FF. ...	400	54.00	—	—	12.00	4.00	30.00	—
57	78	BT. FF. SF. P. ...	400	48.00	1.00	—	18.00	2.00	30.00	1.00
58	79	BT. FF. SF. P. ...	400	62.00	—	—	16.00	—	22.00	—
59	80	BT. FF. SF. ...	400	78.00	—	—	11.00	4.00	7.00	—
60	81	BT. FF. SF. ...	400	49.00	—	—	25.00	4.00	20.50	1.50
61	82	BT. FF. ...	400	52.00	1.00	—	15.00	—	32.00	—
62	83	BT. FF. SF. B. ...	400	61.00	—	—	20.00	2.00	17.00	—
63	84	BT. FF. P. ...	400	39.00	4.00	1.00	14.00	—	42.00	—
64	85	BT. FF. SF. ...	400	66.00	2.00	1.00	12.00	—	19.00	—
65	86	BT. FF. ...	400	75.00	—	—	14.00	—	11.00	—
66	87	BT. FF. ...	400	71.00	—	—	10.00	—	19.00	—
67	88	BT. FF. B. ...	400	71.00	3.00	1.00	13.00	—	10.00	2.00
68	89	BT. FF. ...	400	45.00	4.00	—	20.00	1.00	28.00	2.00
69	90	BT. FF. ...	400	65.00	1.00	—	11.00	—	23.00	—
70	91	BT. FF. B. ...	400	72.00	—	1.00	11.00	—	14.00	2.00
71	92	BT. FF. ...	400	57.00	1.00	—	16.00	7.00	15.00	4.00
72	93	*BT. FF. ...	400	51.00	—	—	17.00	5.00	27.00	—
73	94	*BT. FF. ...	400	74.00	—	—	13.00	—	13.00	—
74	95	*BT. FF. ...	400	60.00	—	—	12.00	5.00	23.00	—
75	96	*BT. FF. ...	400	66.00	6.00	2.00	14.00	—	11.00	1.00
76	97	*BT. FF. ...	400	54.00	1.00	—	16.00	2.00	26.00	1.00
77	98	*BT. FF. B. ...	400	39.00	1.00	—	19.00	4.00	36.00	1.00
78	99	*BT. FF. ...	400	72.00	2.00	—	12.00	—	14.00	—
79	100	*BT. FF. ...	400	66.00	—	—	12.00	1.00	20.00	1.00
80	101	*BT. FF. ...	400	46.00	—	—	15.00	3.00	35.00	1.00
81	102	*BT. FF. ...	400	48.00	5.00	—	14.00	7.00	26.00	—
82	103	Quartan. FF. SF. ...	400	38.00	—	—	12.00	—	50.00	—
83	104	<i>Plasmodium tenue</i> . FF. ...	400	72.00	1.00	—	11.00	2.00	14.00	—
Arithmetic average, Benign Tertian, Serial Nos. 40-81			—	60.25	1.17	0.18	14.46	2.14	21.08	0.71

ABBREVIATIONS

BT. = Benign Tertian

FF. = Fever forms

SF. = Sexual forms

B. = *Basophilia diffusa* or PolychromasiaBp. = *Basophilia punctata*

P. = Pigment in Large Mononuclears

* In these cases malaria was diagnosed upon blood count alone. The parasite was found later—and only after prolonged search.

TABLE II

DIFFERENTIAL BLOOD COUNTS IN CONVALESCENCE FROM MALARIA

Week	Serial No.	Case No.	Remarks, etc.	Poly-morpho-nuclears	Eosino-philic	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
1st week	268	7	4 days later. P.	38.00	1.00	—	23.00	7.00	31.00
	269	8	4 "	37.00	18.00	—	13.00	4.00	28.00
	270	12	4 " MT. FF. B.	51.00	—	—	21.00	13.00	15.00
	271	6	7 "	46.00	13.00	—	14.00	4.00	23.00
	272	10	7 "	60.66	8.66	—	12.00	8.00	10.66
	273	29	7 "	49.00	16.00	—	11.00	4.00	20.00
	274	31	7 " B.	36.50	10.50	—	10.00	1.50	41.50
	275	40	7 " N.	65.33	10.00	—	8.00	0.66	16.00
2nd week	276	35	10 days	38.00	11.00	2.00	19.00	6.00	24.00
	277	36	10 "	70.00	7.00	—	11.00	3.00	9.00
	278	2	14 "	49.50	15.00	1.00	15.00	12.00	7.50
	279	3	14 "	40.00	18.00	—	12.00	9.00	21.00
	280	5	14 "	76.00	5.50	—	8.00	0.50	10.00
	281	7	14 "	69.00	2.00	—	15.00	7.00	8.00
	282	8	14 "	45.50	26.00	1.00	6.50	1.50	19.50
3rd week	283	13	* 18 days	66.00	4.00	—	16.00	7.00	7.00
	284	12	19 "	56.00	3.00	1.50	15.50	3.50	20.50
	—	11	19 " <i>vide</i> Table 2	68.00	4.00	—	15.00	6.00	7.00
	285	14	* 19 "	55.00	5.00	—	15.00	15.00	10.00
	286	15	* 20 "	46.66	8.66	0.66	20.00	12.00	12.00
	287	16	* 21 "	46.00	7.00	—	19.00	14.00	14.00
	288	31	21 " Relapse. BT. FF. SF. B. Sd.	29.00	2.66	—	35.33	5.33	27.66
4th week	289	17	22 days. MT. SF.	69.00	1.00	—	19.00	2.00	9.00
	290	18	22 " Relapse. BT. FF. B. ...	52.00	2.00	2.00	18.00	10.00	16.00
	291	19	* 23 "	64.00	13.00	—	3.00	12.00	8.00
	292	17	25 " Before meals	41.00	3.00	—	22.00	22.00	12.00
	293	—	After meals	65.00	1.00	—	15.00	15.00	4.00
	294	7	27 " N.	46.00	7.00	1.00	11.00	4.00	31.00
	295	11	27 " MT. SF.	51.00	10.00	—	16.00	13.00	10.00
5th week	296	5	32 days. Relapse. BT. FF. Sd. ...	68.00	1.00	1.00	14.00	6.00	10.00
	297	19	34 "	71.00	4.00	1.00	3.00	8.00	13.00
	298	31	35 " 14 days from Relapse ...	51.50	18.50	—	13.00	1.00	16.00
	299	36	35 "	61.50	6.00	—	10.00	—	22.00
	300	41	35 " Relapse. BT. FF. ...	73.00	—	—	14.00	1.00	12.00
6th week	301	12	36 days	39.33	14.66	0.66	12.00	7.33	26.00
	302	18	36 " 14 days from Relapse ...	57.00	6.00	—	10.00	9.00	18.00
	303	8	37 "	40.00	33.00	—	6.00	3.00	18.00
	304	1	42 "	36.66	26.66	1.33	14.00	6.00	15.33
	305	2	42 " Relapse. BT. FF. ...	66.66	2.00	—	14.66	7.33	9.39
	306	7	42 "	56.00	5.00	—	12.00	5.00	22.00
	307	11	42 "	42.00	5.00	—	15.00	19.00	19.00
	308	14	42 "	65.00	12.00	—	11.00	2.00	10.00
	309	15	42 "	61.00	2.50	—	14.00	5.00	17.50
	310	16	42 "	32.00	11.00	—	12.00	10.00	35.00
7th week	—	20	43 days. <i>Vide</i> Table 2	44.00	21.00	1.00	13.00	1.00	20.00
	311	1	49 "	43.00	24.00	5.00	11.00	7.00	10.00
	312	19	49 "	55.00	10.00	—	15.00	4.00	16.00
8th week	313	12	50 days	43.00	22.00	—	12.00	6.00	17.00
	314	17	53 " MT. SF.	74.00	1.00	1.00	14.00	3.00	7.00
	315	30	54 " BT. SF. Marked Leucocytosis	77.50	7.50	—	2.50	1.50	11.00
	316	31	55 " 34 days from Relapse. BT. FF. SF. Sd. No fever	56.00	4.00	—	17.00	6.00	17.00
	317	18	56 " Relapse. BT. FF. ...	46.00	5.00	—	21.00	6.00	22.00

TABLE 11.—(Continued)

Week	Serial No.	Case No.	Remarks, etc.	Poly-morpho-nuclears	Eosino-philes	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes
9th week	318	20	59 days. Relapse. MT. FF. SF. Bp.	67.00	6.00	—	14.00	7.00	6.00
	319	7	63 " " " " " " " " " "	62.00	—	—	11.00	4.00	23.00
	320	18	63 " 7 days from Relapse ...	32.00	5.00	—	33.00	12.00	18.00
10th week	321	11	65 days. Relapse. MT. FF. SF. B. Infection 1/500 r.b.cs.	43.00	2.00	—	22.00	9.00	24.00
	322	13	65 " " " " " " " " " "	64.00	10.00	2.00	10.00	3.00	11.00
	323	2	66 " Relapse. BT. FF. Bp. Sd. 24 days from last Relapse	76.00	—	1.00	13.00	4.00	6.00
	324	14	66 " " " " " " " " " "	56.00	8.00	—	12.00	5.00	19.00
	325	16	67 " Bp. " " " " " " " "	56.00	5.00	—	10.00	6.00	23.00
11th week	326	15	75 days	52.00	11.00	0.50	6.50	0.50	29.50
12th week	327	6	80 days	51.00	9.00	—	10.00	12.00	18.00
13th week	328	5	88 days	38.00	25.00	—	8.00	7.00	22.00
	329	8	89 " " " " " " " " " "	42.00	20.00	—	5.00	3.00	30.00
15th week	330	13	103 days	70.00	11.00	—	3.00	—	16.00
16th week	331	12	106 days	58.00	7.00	—	6.00	6.00	22.00
	332	20	110 " 51 days from Relapse ...	64.00	6.00	—	9.33	7.33	13.33
	333	7	110 " " " " " " " " " "	77.00	3.00	—	4.00	4.00	12.00
	334	31	112 " 91 days from Relapse ...	59.50	16.00	—	2.00	2.00	20.50
17th week	335	2	115 days. 49 days from Relapse ...	54.00	8.00	—	13.00	12.00	13.00
18th week	336	11	123 days. SF.	57.00	3.00	—	6.00	7.00	27.00
	337	16	125 " " " " " " " " " "	47.00	16.00	—	10.00	5.00	22.00
	338	2	126 " 60 days from Relapse ...	50.00	2.00	—	9.00	12.00	27.00
19th week, etc.	339	1	131 days. Megalocytes	58.00	14.00	—	4.00	5.00	19.00
	340	17	133 " Bp.	70.00	6.00	—	5.00	3.00	16.00
	341A	2	172 " Pneumonia. <i>Vide</i> Table 5 ...	82.00	—	—	6.00	2.00	10.00
	341	30	182 " " " " " " " " " "	50.00	4.66	—	3.33	6.00	36.00
	342	29	232 " " " " " " " " " "	69.00	11.00	1.00	1.00	1.00	17.00
	343	10	233 " " " " " " " " " "	46.00	25.00	1.00	7.00	3.00	18.00
	344	6	250 " " " " " " " " " "	61.00	11.00	—	3.00	7.00	18.00
	345	9	253 " " " " " " " " " "	47.00	6.00	—	—	1.00	46.00
		12	255 " Bronchitis. <i>Vide</i> Table 7 ...	73.00	1.00	—	3.00	3.00	20.00
		14	280 " " " " " " " " " "	78.00	1.00	—	—	4.00	17.00
	346	16	296 " " " " " " " " " "	61.00	9.00	—	12.00	7.00	11.00
	347	19	298 " " " " " " " " " "	55.00	7.00	—	6.00	1.00	30.00
	348	15	303 " " " " " " " " " "	71.00	7.00	—	1.00	1.00	20.00

NEW ABBREVIATIONS.—Sd. = Schüffner's dotting especially marked.

* Benign Tertian parasites were found during primary attack, but no count was then made.

TABLE 12

THE LARGE MONONUCLEAR AVERAGES OF THE PREVIOUS TABLE ARE SET OUT BELOW IN PROGRESSIVE WEEKS. WHERE A RELAPSE OCCURS, THIS IS INDICATED WITH THE INITIAL 'R,' AND THE COUNT OF THAT DATE STARTS AGAIN AT 'FIRST DAY OF FEVER.'

Case No.	Day, 1st	Wk. 1	Wk. 2	Wk. 3	Wk. 4	Wk. 5	Wk. 6	Wk. 7	Wk. 8	Wk. 9	Wk. 10	Wk. 11	Wk. 12	Wk. 13	Wk. 14	Wk. 15	Wk. 16	Wk. 17	Wk. 18	Wk. 19, etc.
1	14 ³ / ₈	—	—	—	—	—	14	11	—	—	—	—	—	—	—	—	—	—	—	4
2	10	—	15	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—
	14 ³ / ₈	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	13	—	—	—	—	—	—	13	9	—	—	—	—	—	—	—	—	—	—	—
3	14	—	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5	18	—	8	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—
	14	—	—	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	—	—
6	15	14	—	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	3
7	26 ¹ / ₂	23	15	—	11	—	12	—	—	11	—	—	—	—	—	—	4	—	—	—
8	8	13	6 ¹ / ₂	—	—	—	6	—	—	—	—	—	—	5	—	—	—	—	—	—
9	22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
10	19	12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7
11	20 ¹ / ₂	—	—	15	16	—	15	—	—	—	R	—	—	—	—	—	—	—	—	—
	22	—	—	—	—	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—
12	—	21	—	15 ¹ / ₂	—	—	12	—	12	—	—	—	—	—	—	—	6	—	—	3
13	—	—	—	16	—	—	—	—	—	—	10	—	—	—	—	3	—	—	—	—
14	—	—	—	15	—	—	11	—	—	—	12	—	—	—	—	—	—	—	—	0
15	—	—	—	20	—	—	14	—	—	—	—	6 ¹ / ₂	—	—	—	—	—	—	—	1
16	—	—	—	19	—	—	12	—	—	—	10	—	—	—	—	—	—	10	12	—
17	—	—	—	—	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
					22	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
					15	—	—	—	14	—	—	—	—	—	—	—	—	—	—	5
18	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18	—	10	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	21	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19	—	—	—	—	3	3	—	15	—	—	—	—	—	—	—	—	—	—	—	6
20	20 ¹ / ₂	—	—	—	—	—	—	13	—	R	—	—	—	—	—	—	—	—	—	—
	14	—	—	—	—	—	—	—	9 ¹ / ₃	—	—	—	—	—	—	—	—	—	—	—
29	24	11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
30	15	—	—	—	—	—	—	—	*2 ¹ / ₂	—	—	—	—	—	—	—	—	—	—	3 ¹ / ₈
31	10	10	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	35 ¹ / ₃	—	—	13	—	17	—	—	—	—	—	—	—	2	—	—	—	—	—	—
35	18	19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	12 ² / ₃	—	11	—	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
40	10	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
41	12	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The Arithmetic Averages for first day of fever in the above table, which includes a few counts from Table 10, and for successive weeks are as follows:—

1st day of fever	Observations	27	...	Average	16.88
1st week of Convalescence	"	10	...	"	16.40
2nd	"	"	"	7	...	"	11.07
3rd	"	"	"	7	...	"	16.21
4th	"	"	"	6	...	"	14.33
5th	"	"	"	3	...	"	10.00
6th	"	"	"	8	...	"	12.00
7th	"	"	"	4	...	"	13.00
8th	"	"	"	5	...	"	10.47*
9th	"	"	"	2	...	"	8.50
10th	"	"	"	3	...	"	10.06
11th	"	"	"	1	...	"	6.50
12th	"	"	"	1	...	"	10.00
13th	"	"	"	2	...	"	3.50
14th	"	"	"	0	...	"	—
15th	"	"	"	1	...	"	3.00
16th	"	"	"	2	...	"	5.00
17th week and onwards	"	13	...	"	4.18

* Excluding 2¹/₂.

TABLE 13

DIFFERENTIAL BLOOD COUNTS FROM WHICH MALARIA WAS DIAGNOSED

Serial No.	Case No.	Remarks, etc.	Cells counted	Poly-morpho-nuclears	Eosino-philic	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes	Broken nuclei
238	11	—	46.50	6.50	—	20.50	—	26.50	—
239	11	19 days later	—	68.00	4.00	—	15.00	6.00	7.00	—
		27 " " MT. SF. ...	—	51.00	10.00	—	16.00	13.00	10.00	—
		42 " "	—	42.00	5.00	—	15.00	19.00	19.00	—
		65 " " MT. FF. SF. ...	—	43.00	2.00	—	22.00	9.00	24.00	—
		B. Infection 1/500								
		123 " " MT. SF. ...	—	57.00	3.00	—	6.00	7.00	27.00	—
240	20	—	56.00	2.75	0.25	20.50	—	20.50	—
241		43 days later	—	44.00	21.00	1.00	13.00	1.00	20.00	—
		58 " " MT. FF. SF. ...	—	67.00	6.00	—	14.00	7.00	6.00	—
		Bp.								
		109 " "	—	64.00	6.00	—	9.33	7.33	13.33	—
242	21	4 days after fever. B. P.	—	53.00	11.00	—	17.00	16.00	3.00	—
243	22	4 " " " B. P.	—	61.00	—	—	13.00	8.00	18.00	—
244		15 " " "	—	69.00	6.00	—	13.00	—	12.00	—
245	23	17 " " "	—	64.00	5.00	1.00	15.00	5.00	10.00	—
246	24	23 " " "	—	40.00	39.33	0.66	8.00	6.00	5.99	—
247	25	32 " " "	—	38.00	17.00	—	23.00	12.00	10.00	—
248		50 " " "	—	48.00	11.00	—	16.00	8.00	17.00	—
249	26	36 " " "	—	42.00	4.00	1.00	16.00	22.00	15.00	—
250		66 " " "	—	46.66	16.66	—	11.33	6.00	19.33	—
251	27	41 " " "	—	47.00	25.00	—	14.00	11.00	3.00	—
252		63 " " "	—	49.00	13.00	—	15.00	8.00	15.00	—
253	28	48 " " "	—	43.00	6.00	1.00	16.00	11.00	23.00	—
254	32	Bp.	—	60.66	1.33	—	18.66	2.66	16.66	—
255		3 days after fever	—	56.00	3.00	—	18.00	12.00	11.00	—
256	33	4 " " " B. Bp.	—	29.00	1.00	—	18.00	10.00	42.00	—
257	34	P.	—	41.00	—	—	19.00	13.00	27.00	—
		200 days later. BT. FF. SF.	—	58.50	10.00	0.50	16.50	1.00	13.50	—
258	35	—	33.00	6.00	—	15.00	15.00	31.00	—
		32 days later. MT. FF. ...	—	54.00	3.00	1.00	18.00	2.00	22.00	—
		10 days after fever	—	38.00	11.00	2.00	19.00	6.00	24.00	—
259	39	—	64.00	2.00	—	19.00	5.00	10.00	—
260	105	N.A.D.	200	44.00	14.00	—	13.50	1.00	27.00	0.50
		1 day after; BT. FF.								
261	106	400	52.00	1.00	—	16.50	8.00	22.50	—
262	107	400	76.00	1.00	—	13.00	—	10.00	—
263	108	400	61.00	—	1.00	16.00	2.00	20.00	—
264	109	P.	400	47.00	—	—	15.00	1.00	37.00	—
265	110	P.	400	70.00	—	—	11.00	—	19.00	—
266	111	*	400	72.00	0.50	—	10.50	1.50	15.50	—
267	112	400	67.00	—	—	11.00	3.50	17.00	1.50
Arithmetic average of all cases diagnosed on counts			30	52.93	7.27	0.19	15.34	6.49	17.70	0.07
Average of cases relapsing later			12	52.71	5.94	0.10	15.46	5.08	20.67	0.04
" " left			18	53.07	8.16	0.26	15.28	7.43	15.72	0.08

Serial Numbers 238-260 were counts from Gurkhas, remainder from Sikhs, Jats, etc.

ABBREVIATIONS

MT. = Malignant Tertian

FF. = Fever forms

B. = *Basophilus diffusa*

BT. = Benign Tertian

SF. = Sexual forms

Bp. = *Basophilus punctata*

P. = Pigment in Large Mononuclears

N.A.D. = No appreciable disease

* I was informed afterwards that this case had malaria 23 days earlier.

TABLE 14

DIAGNOSIS BY BLOOD COUNT DURING AND AFTER AN EPIDEMIC

Serial No.	Case No.	Date of admission	Diagnosis returned	My diagnosis from symptoms spleen, chart, etc.	Independant blood findings	Large mono-nuclears	
31	M1	Mar. 5	Malaria ...	Malaria ...	BT. FF. B. ...	12.00	
32	M2	" 3	" ...	" ...	BT. FF. ...	12.00	
349	M3	" 3	" ...	No appreciable disease	2.00	
350	M4	" 3	" ...	Adenitis of neck ...	Leucocytosis ...	—	
351	M5	" 3	" ...	" "	1.00	
33	M6	" 3	" ...	Malaria ...	BT. SF. P. ...	12.66	
34	M7	" 2	" ...	" ...	BT. SF. P. ...	11.50	
39	M8	" 2	" ...	" ...	MT. SF. B. ...	11.00	
352	M9	" 2	" ...	" ...	B. P. ...	11.50	
353	M10	" 1	" ...	No evidence of malaria. C.	2.00	
354	M11	Feb. 27	" ...	Malaria, typical chart	15.50	
355	M12	" 27	" ...	No evidence of malaria. C.	3.50	
356	M13	" 25	" ...	Malaria	14.00	
357	M14	" 25	Gumboil ...	Gumboil	0.50	
358	M15	" 25	Dyspepsia ...	Dyspepsia	2.50	
359	M16	—	Sick attendant	0.50	
360	M17	—	" "	1.50	
361	M18	Feb. 23	Malaria ...	Chart atypical	2.00	
362	M19	" 23	" ...	" missing	10.80	
363	M20	" 23	" ...	" atypical	4.00	
364	M21	" 20	" ...	" typical	10.50	
365	M22	" 17	" ...	" "	11.00	
366	M23	" 15	" ...	" "	10.00	
367	M24	" 13	" ...	Only admitted 2 days	0.66	
368	M25	" 8	" ...	Chart typical	10.00	
369	M26	" 6	" ...	" atypical ...	Leucocytosis ...	2.75	
370	M27	" 4	" ...	" typical	11.00	
371	M28	" 3	" ...	" lost	12.00	
372	M29	" 1	" ...	" atypical	3.00	
373	M30	Jan. 29	" ...	" "	1.00	
374A	M31	" 27	" ...	" typical. Relapsing ...	BT. FF. SF. ...	8.00	
374	M32	" 24	" ...	" "	16.00	
375	M33	" 21	" ...	" "	10.50	
376	M34	" 21	" ...	" atypical	5.00	
377	M35	" 21	" ...	Only 3 days' admission	4.00	
378	M36	" 21	" ...	Chart lost	3.00	
379	M37	" 21	" ...	" atypical	4.50	
380	M38	" 20	" ...	" typical ...	Bp. ...	13.00	
381	M39	" 19	" ...	" "	13.00	
382	M40	" 19	" ...	" "	16.50	
383	M41	" 18	" ...	" " ...	B. Bp. ...	18.50	
384	M42	Jan. 4	Diarrhoea	4.00	
385	M43	" 2	" ...	Chart typical. Relapsing ...	BT. FF. SF. P. ...	18.50	
386	M44	Dec. 26	" ...	" "	12.00	
387	M45	" 26	" ...	" "	10.50	
388	M46	" 25	" ...	" " 9 days' fever	18.00	
389	M47	" 25	" ...	" "	17.00	
390	M48	" 23	" ...	Only admitted 1 day	3.00	
391	M49	" 22	" ...	Chart lost	5.00	
392	M50	" 20	" ...	" typical	9.00	
393	M51	" 19	" ...	" "	10.00	
394	M52	" 17	" ...	" "	9.50	
395	M53	" 16	" ...	" "	22.00	
37	M54	" 14	" ...	" " Relapsing ...	BT. FF. SF. ...	15.00	
396	M55	" 14	" ...	" "	9.33	
397	M56	" 10	" ...	" atypical	3.00	
38	59	—	NO PREVIOUS ADMISSIONS			BT. FF. P. ...	17.50
260	105	—	Admitted next day with BT.	13.50	

C. = Convalescent.

TABLE 14.—(Amplified.)

Serial No.	Case No.	Remarks, etc.	Cells counted	Poly-morpho-nuclears	Eosino-philic	Mast cells	Large mono-nuclears	Large lympho-cytes	Small lympho-cytes	Broken nuclei
31	M1	BT. FF. B. ...	200	74.50	—	0.50	12.00	3.00	8.50	1.50
32	M22	BT. FF. ...	100	65.00	4.00	—	12.00	4.00	13.00	2.00
349	M3	100	57.00	15.00	1.00	2.00	5.00	20.00	—
350	M4	Leucocytosis ...	200	74.50	12.50	—	—	—	13.00	—
351	M5	200	72.00	4.50	—	1.00	2.00	20.50	—
33	M6	BT. SF. P. ...	150	42.00	2.00	—	12.66	3.33	39.33	0.66
34	M7	BT. SF. P. ...	200	64.00	5.00	—	11.50	1.50	16.50	0.50
39	M8	MT. SF. B. ...	100	42.00	4.00	—	11.00	1.00	42.00	—
352	M9	B. P. ...	200	46.00	4.50	—	11.50	1.50	36.50	—
353	M10	200	58.00	7.00	—	2.00	1.50	30.00	1.50
354	M11	200	64.00	2.50	—	15.50	3.50	12.00	2.50
355	M12	200	74.00	0.50	0.50	3.50	1.50	19.50	0.50
356	M13	200	54.00	5.00	0.50	14.00	4.50	21.50	0.50
357	M14	200	45.00	13.50	—	0.50	2.50	38.50	—
358	M15	200	65.00	8.50	—	2.50	2.50	23.00	—
359	M16	Sick attendant ...	200	62.50	11.00	—	0.50	1.00	25.00	—
360	M17	" " ...	200	73.50	3.50	—	1.50	1.00	20.50	—
361	M18	200	59.00	9.00	—	2.00	2.00	27.00	1.00
362	M19	250	48.80	14.00	1.20	10.80	1.20	24.00	—
363	M20	100	56.00	13.00	—	4.00	2.00	25.00	—
364	M21	200	47.00	13.50	0.50	10.50	1.50	27.00	—
365	M22	200	54.50	11.00	—	11.00	0.50	23.00	—
366	M23	200	66.50	1.00	—	10.00	—	22.50	—
367	M24	150	42.66	22.00	1.33	0.66	0.66	32.66	—
368	M25	200	53.00	11.00	—	10.00	2.00	24.00	—
369	M26	Leucocytosis ...	400	59.00	0.50	0.25	2.75	0.50	36.00	1.00
370	M27	200	42.50	30.00	1.00	11.00	1.50	13.50	0.50
371	M28	200	71.00	1.00	—	12.00	1.00	15.00	—
372	M29	100	72.00	6.00	—	3.00	2.00	17.00	—
373	M30	100	57.00	15.00	—	1.00	1.00	26.00	—
35	M31	BT. FF. SF. ...	100	60.00	1.00	—	8.00	—	31.00	—
374	M32	200	50.00	6.00	—	16.00	6.00	21.00	1.00
375	M33	200	62.50	3.50	0.50	10.50	0.50	22.50	—
376	M34	200	57.00	9.00	—	5.00	0.50	28.50	—
377	M35	200	59.50	6.50	—	4.00	1.50	28.50	—
378	M36	200	63.00	14.00	—	3.00	—	20.00	—
379	M37	200	52.50	12.00	—	4.50	5.00	26.00	—
380	M38	Bp. ...	200	40.00	25.00	1.00	13.00	—	21.00	—
381	M39	100	60.00	4.00	—	13.00	2.00	19.00	2.00
382	M40	200	35.00	11.00	—	16.50	4.00	32.50	1.00
383	M41	B. Bp. ...	200	61.50	6.00	—	18.50	1.50	12.50	—
384	M42	200	57.00	11.50	—	4.00	1.50	26.00	—
385A	M43	BT. FF. SF. P. ...	200	59.00	3.00	—	18.50	0.50	18.50	0.50
386	M44	200	62.00	13.00	1.00	12.00	1.00	10.00	1.00
387	M45	200	47.00	16.00	0.50	10.50	0.50	24.50	1.00
388	M46	200	53.00	4.00	0.50	18.00	3.50	19.50	1.00
389	M47	200	52.00	13.00	—	17.00	—	17.00	1.00
390	M48	Leucopenia ...	100	46.00	19.00	1.00	3.00	1.00	30.00	—
391	M49	200	47.00	9.00	—	5.00	1.00	38.00	—
392	M50	200	47.00	18.00	1.00	9.00	—	25.00	—
393	M51	100	49.00	20.00	1.00	10.00	1.00	19.00	—
394	M52	200	50.00	13.00	—	9.50	2.50	24.50	0.50
395	M53	100	35.00	7.00	—	22.00	9.00	26.00	1.00
37	M54	BT. FF. SF. ...	200	54.00	7.00	1.00	15.00	—	23.00	—
396	M55	300	32.00	6.33	—	9.33	0.33	52.00	—
397	M56	200	47.00	20.00	—	3.00	1.00	29.00	—

The illness in the case of Nos. M42-M56, below the line, occurred more than 8 weeks from the date the slide was taken (i.e., March 5).

REFERENCES

- STOTT, H. (1915). Studies in Malaria, Part III. *Ind. Med. Gaz.*, L, pp. 85-91. (See p. 88.)

ON THREE NEW AFRICAN MIDGES

BY

HENRY F. CARTER, F.E.S.

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PLATE III

The three minute Diptera described below are members of the family *Chironomidae*, or Midges, and further, are all referable to that section, the sub-family *Ceratopogoninae*, which contains the great majority of the biting or blood-sucking midges at present known. Whether the species considered in this paper are possessed of phlebotomic habits cannot yet be definitely stated. Two of them, however, belong to a genus (*Culicoides*) notorious for its inclusion of some most annoying pests, while the formation of the mouth parts in the species of *Forcipomyia* shows it to be entirely capable of indulging in such tastes. The types and co-types of the species herein described have been deposited in the Museum of the Liverpool School of Tropical Medicine.

Genus *Forcipomyia*, Meig.

Forcipomyia lejanui, sp. n.

(Plate III, figs. 1, 2 and 3)

A minute blackish brown fly, with yellowish brown legs and grey unspotted wings, which are slightly longer than the abdomen.

Head dark brown, the vertex bearing sparsely arranged, short golden brown hairs. *Eyes* bare, narrowly separated in the middle line. *Proboscis* as long, or nearly as long, as the head; labium brownish black with relatively large labellae, and furnished with several long dark hairs. *Clypeus* dark chestnut brown, bearing a few dark, forwardly projecting hairs. *Palpi* (fig. 1) dark brown, with rather coarse hairs; third joint much swollen, the orifice of the sense organ situated at the apex of a conspicuous tubercle; apical segment with four or five relatively long hairs. *Antennae*, except the large second segment, paler in colour than the palpi, with

yellowish brown hairs; segments 3-10 very short, globose (3.7 slightly broader than long); their lengths combined considerably less than the total length of the five elongate terminal joints; last segment with a small but distinct terminal stylet.

Thorax shining black or blackish brown, markedly convex dorsally, and with a relatively large depressed posterior area sloping abruptly to the scutellum; sparsely clothed with short golden brown hairs, intermixed with longer black ones laterally and posteriorly. *Pleurae* dark brown. *Scutellum* coloured similarly to the thorax, with four pairs of very long, golden brown bristles and several short hairs arising from the posterior margin.

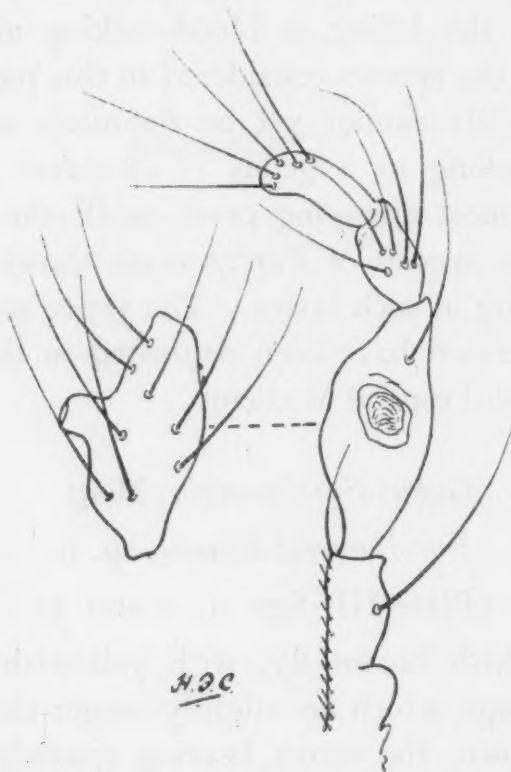


FIG. 1. Palpus of *Forcipomyia lejanui*, n.sp. ♀. ($\times 400$ circa.)

Abdomen dull blackish brown dorsally, with numerous dark hairs which become somewhat longer laterally and apically. *Venter* slightly paler in colour, with pale brown hairs.

Wings (Pl. III, fig. 5) densely clothed with relatively long decumbent hairs. Venation as shown in fig. 5; space between separated portions of first and third longitudinal veins extremely narrow and difficult to distinguish; petiole and bases of upper and

lower rami of fourth longitudinal vein indistinct. *Halteres* of a creamy white colour.

Legs yellowish brown, rather densely haired. Femora unarmed. Fore tibiae each with a short, stout apical ventral spine; hind tibiae each with several long hairs on the dorsal surface, a ventral spur or spine, and a double transverse row of short bristles at the apex; the outermost row is apparently composed of six relatively stout hairs, and the innermost of from twelve to fourteen shorter and more delicate ones. First tarsal segment of hind legs (Pl. III, fig. 2) about twice the length of the succeeding joint. Empodium hairy and conspicuous, almost as long as the claws.

Length 1.50 mm.; length of wing 0.8 mm.

Habitat: Salaga, Gold Coast. Dr. G. E. H. Lefanu, 11.6.1911; six females.

The exact generic position of this interesting little fly is at present somewhat uncertain. In accordance with the recent definitions of the Ceratopogonine genera given by Malloch, it would seem to belong either to his new genus *Pseudoculicoides* or to *Forcipomyia*, Meig. *Ceratopogon*, Meig., in its narrow sense may be eliminated, owing to the presence, in this species, of distinct decumbent hairs on the greater part of the wing field. The species of *Pseudoculicoides* differ from those of *Forcipomyia*, *inter alia*, in possessing rather sparsely-arranged hairs on the wings, indistinct empodia, and longer basal joints to the hind tarsi. In spite of the latter character, however, the species under consideration apparently possesses closer affinities to the members of Meigen's genus, and has, therefore, been placed accordingly.

Genus *Culicoides*, Latr.

A considerable number of minute flies belonging to this genus were collected by Professor J. W. W. Stephens in the region of the Gizeh Pyramids during December, 1909. On examining the material, three distinct and easily separable species were discovered, of which two are new, and are described below. The third species, forming by far the greater part of the collection, was the common European *C. varius*, Winn., which, it would seem, has not previously been recorded from Africa.

Culicoides cordiformitarsis, sp. n.

(Plate III, figs. 3 and 4)

A small dark brown species with yellowish brown legs and whitish wings, on each of which is a single conspicuous dark brown spot situated near the middle of the anterior margin. *Fourth tarsal segment of all the legs heart shaped.*

Head: Vertex dark brown, clothed with short golden brown hairs, front dark ashy grey. *Eyes* bare, not contiguous. *Proboscis* as long as the head; labium dark brown, with short yellowish hairs. *Clypeus* dark brown, with several relatively long brown hairs projecting over the base of the proboscis. *Palpi* dark, clothed with rather long yellowish or golden hairs; third segment slightly incrassate. *Antennae* yellowish brown, with whorls of comparatively short golden hairs; second segment (first of some authors) dark brown, becoming greyish pollinose apically, bearing a few short pale hairs; segments 3-10 sub-cylindrical, rather longer than wide.

Thorax dark brown, humeral region and a ring round each anterior depression markedly light grey pollinose; sparsely clothed with short golden hairs. *Scutellum* dark brown, with apparently four long border bristles and several shorter paler hairs. *Pleurae* dark brown.

Abdomen dull dark brown, clothed with numerous, somewhat scattered golden brown hairs, which are longer laterally and apically; at the extreme apex several long dark brown hairs are intermixed with the paler ones.

Wings (Pl. III, fig. 4) with venation and ornamentation as shown in the figure. The very pale coloration of the wing field renders the dark brown spot on the second cell (formed by the first and third longitudinal veins at the anterior margin) very conspicuous. Petiole and bases of upper and lower branches of the fourth longitudinal vein indiscernible. Wings clothed with short pale hairs on the apical two-thirds. *Halteres* with white knobs and yellowish stems.

Legs yellowish brown, the coxae and trochanters darker. Hind tibiae with four or five very long brownish hairs on the outer side, a short, strong, black, apical spur ventrally situated, and a

transverse row of six long and two short bristles immediately below the apex. Tarsi all densely hairy, the hairs golden brown and black intermixed, the paler ones predominating; basal joint of hind tarsus more than twice the length of the second segment; fourth tarsal segment (Pl. III, fig. 3) in all the legs very characteristic, being small and cordiform. Empodium minute, about one-sixth the length of the claws.

Length 2.00 mm.; *length of wing* 2.00 mm.

Habitat: Gizeh Pyramids, near Cairo, Egypt. Prof. J. W. W. Stephens, December, 1909; one female.

Culicoides stephensi, sp. n.

(Plate III, fig. 6)

A dark grey midge, with brown legs and conspicuously brown-spotted wings.

Head dark brown, with scattered golden brown hairs on the vertex—laterally, on the same region, are some longer, darker ones, projecting over the eyes. *Eyes* bare, narrowly separated above. *Proboscis* as long as the head, labium dark brown, with scattered golden hairs. *Clypeus* dark chestnut brown, bearing a few moderately long brown hairs. *Palpi* reddish brown, slender, bearing rather pale brown hairs, which are more numerous at the apex. *Antennae*, except the large second segment, yellowish brown, with whorls of pale brown hairs, second segment dark reddish brown, becoming somewhat greyish pollinose towards the apex; segments 3-10 more elongate than usual; segments 5-10, inclusive, being from two to two and a half times as long as broad; five terminal segments elongate cylindrical, the apical joint longer and wider than any of the preceding, their combined lengths approximately equal to the lengths of segments 5-10 taken together.

Thorax. Anterior two-thirds ashy grey, paler grey posteriorly, with three narrow, black longitudinal stripes extending to the scutellum; humeral region, and a narrow line reaching to a spot immediately in front of the anterior depression, light grey pollinose; on each side of this line is a small diffuse blackish grey area; anterior depressions relatively large, shining black. In one

specimen, three distinct oval dark brown spots are visible on the disc; one of these is situated towards the anterior extremity of the median stripe, the others each near the middle of one of the lateral stripes. Dorsum clothed with sparsely arranged pale golden hairs, intermixed with short black ones; immediately before the scutellum are two stout black bristles and a few moderately conspicuous black hairs. *Scutellum* brownish grey, with four border bristles. *Pleurae* dark brown. *Post-scutellum* dark brown, with light grey median and lateral areas anteriorly.

Abdomen dark brown, with short scattered golden brown hairs, which increase in length towards the apical extremity, hind margins of segments narrowly grey. *Venter* similar to dorsum.

Wings (Pl. III, fig. 6) light grey with numerous irregular brown spots, and sparsely clothed with short pale hairs. Veins yellowish brown, venation and ornamentation as in fig. 6. *Halteres* creamy white.

Legs brown, with yellowish knee spots, clothed somewhat thickly with pale brown hairs. Fore tibiae each with a short apical ventral spine; hind tibiae each with a short, stout black ventral spur, and a double transverse row of bristles at the apical extremity, the more distal row is composed of six relatively large and stout bristles, the other of some eighteen shorter and more delicate ones. Basal joints of hind tarsi not quite twice the length of the second segments. Empodium indistinguishable.

Length 1.75 mm.; length of wing 1.70 mm.

Habitat: Gizeh Pyramids, Egypt. Prof. J. W. W. Stephens (to whom I have much pleasure in dedicating this species), December, 1909; three females.

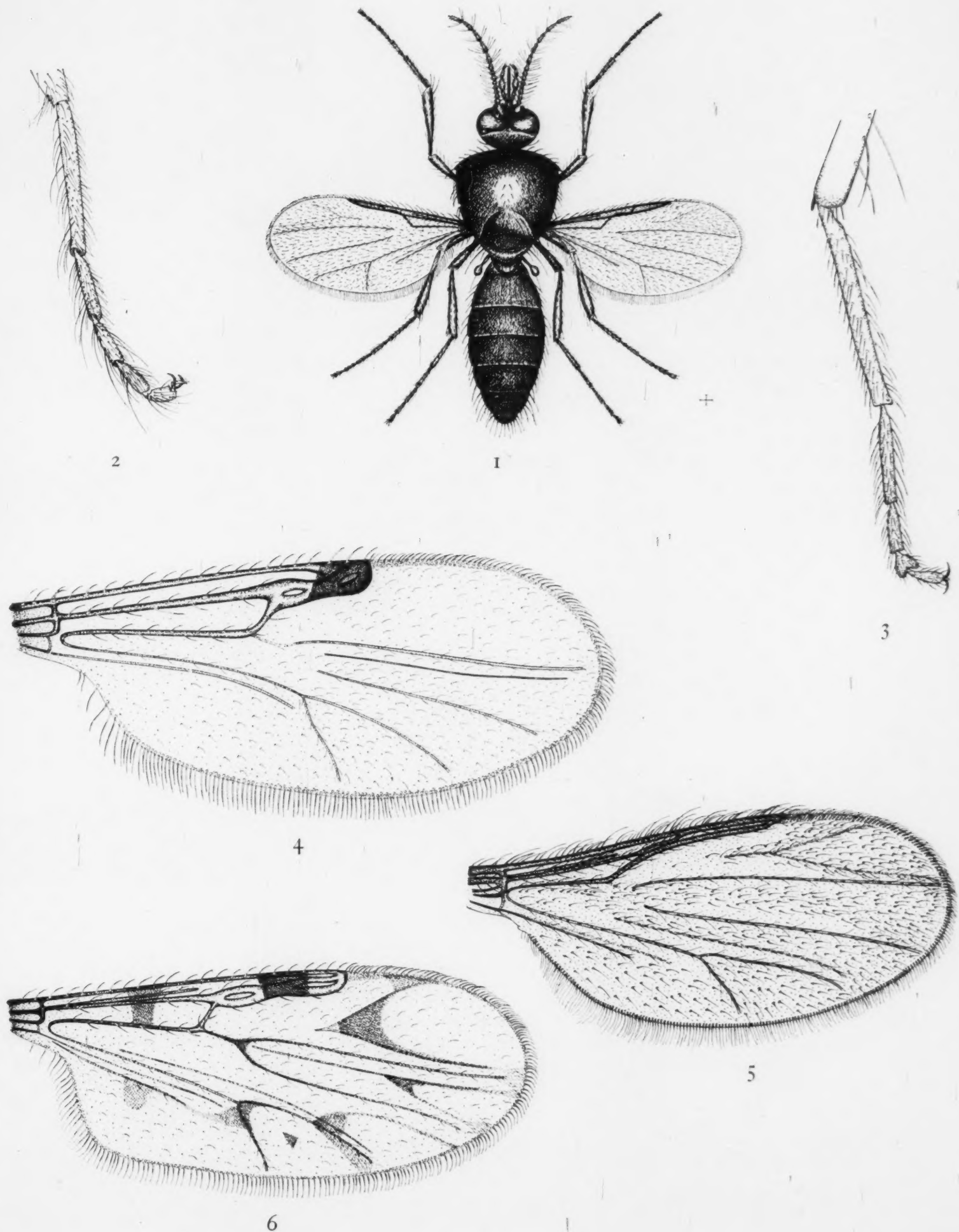
This species evidently bears a marked superficial resemblance to *Ceratopogon* (? *Culicoides*) *puncticollis*, Becker, which occurs at Alexandria. It approaches Becker's species more especially in regard to the ornamentation of the wings, but may be easily separated therefrom by the venation and the apparently complete absence of an empodium. In *C. stephensi* the first and third longitudinal veins separate distally, forming by means of the small cross vein two distinct loops, whereas in *C. puncticollis* these veins are fused throughout their entire length.

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EXPLANATION OF PLATE

- Fig. 1. *Forcipomyia lefanui*, sp. n. female ($\times 40$ circa). Natural size indicated by cross lines.
- Fig. 2. Hind tarsus of *Forcipomyia lefanui*, sp. n., female ($\times 110$ circa).
- Fig. 3. Hind tarsus of *Culicoides cordiformitarsis*, sp. n., female ($\times 110$ circa).
- Fig. 4. Wing of *Culicoides cordiformitarsis*, sp. n., female ($\times 48$ circa).
- Fig. 5. Wing of *Forcipomyia lefanui*, sp. n., female ($\times 90$ circa).
- Fig. 6. Wing of *Culicoides stephensi*, sp. n., female ($\times 48$ circa).



FURTHER WORK ON THE REDUCTION OF THE ALKALINITY OF THE BLOOD IN CHOLERA; AND SODIUM BICARBONATE INJECTIONS IN THE PREVENTION OF URAEMIA*

BY

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Early in 1915, in conjunction with Captain Shorten, I.M.S., I recorded estimations of the alkalinity of the blood in twenty cases of cholera, demonstrating its nearly constant reduction to well below the normal and showing that in cases of post-choleraic uraemia it reached the extreme degrees of from N/100 to N/240, thus confirming by accurate estimations the statement of Sellards (1910, 1914) that this dangerous condition is essentially an acidosis. On my collaboration with Captain Shorten being interrupted by the war, Dr. Satis Chandra Banerjee, to whom I am very greatly indebted for his careful observations, kindly undertook the estimations of the alkalinities of the blood in cholera cases in my ward. In a recent paper (1915) on the results of my system of treatment in 1,000 cases of cholera, I gave a table briefly summarising the results of the test in sixty-two cases. As the number has now risen to upwards of one hundred spread over a complete year, and consequently

* With a 'Note on the Technique of the Estimations of the Alkalinity of the Blood,' by Satis Chandra Banerjee, L.M.S. See page 150.

including all degrees of severity, I propose in the present paper to analyse the records more fully, and to record the results of the routine administration of alkalies intravenously from an early stage of the disease in combating the condition, and their effect in greatly reducing the mortality from the dreaded post-choleraic uraemia.

Table I shows the degree of reduction of the alkalinity of the blood before the administration of alkalies in 104 unselected cases of cholera, taken, in nearly all of them, on admission to hospital. The higher proportion with alkalinities above N/45 in this series, as compared with the series of 62 cases recorded in the paper already referred to, is due to the additional cases having mostly occurred in the rainy season, when the mildest type of cholera prevails in Calcutta.

TABLE I.—The Degrees of Reduction of the Alkalinity and the Causes of Death in 104 Cholera cases.

Alkalinity	Cured	DIED			Total deaths	Total cases	Percentage of deaths	Percentage
		Collapse	Uraemia	Other causes				
Over N/45 ...	21	1	1	1	3	24	12.5	23.1
N/45-N/60 ...	21	3	—	—	3	24	12.5	23.1
N/60-N/80 ...	21	2	1	—	3	24	12.5	23.1
N/80-N/100 ...	16	4	1	1	6	22	27.3	21.1
N/100 and less ...	4	2	4	—	6	10	60.0	9.6
Totals ...	83	12	7	2	21	104		

The data in Table I show that in 77 per cent. of cholera cases the alkalinity of the blood is reduced to N/45 and under, while in no less than 53.9 per cent. it was N/60 and under, while in 30.7 per cent. it reached the extreme degrees of from N/80 to N/240. Moreover, when the alkalinity fell to N/80 and under, the death rate rose markedly from 12.5 to 27.3 per cent., and in cases with alkalinities of 100 and under it reached the very high figure rate of 60 per cent. On turning to the causes of death, it appears that

when the alkalinity falls to N/80, and under, the proportion of deaths in the collapse stage greatly increases, indicating that there is a close relationship between the degree of reduction of the alkalinity and the severity of the disease, quite apart from the question of uraemic complication. Further, when the alkalinity falls to N/100, and below, the death rate from uraemia rises to an extremely high figure, namely, 40 per cent. of the cases, and would have been still higher but for the injections of alkalies as described below.

In cases admitted in the acute stage the specific gravity of the blood on arrival is the best test of the severity of the disease, as it indicates the amount of fluid which has been lost from the body; 1063, 1064, and 1065 pointing to an approximate loss of three, four, and five pints respectively in a subject not previously anaemic. Table II shows a progressively larger proportion of cases with the

TABLE II.—The Relationship of the Specific Gravity of the Blood on admission and the Alkalinity.

Alkalinity	Specific gravity below 1060			Specific gravity 1060-1063			Specific gravity 1064-1065			Specific gravity over 1065		
	Cured	Died	Total	Cured	Died	Total	Cured	Died	Total	Cured	Died	Total
Over N/45 ...	4	—	4	8	1	9	5	1	6	4	1	5
N/45-N/60 ...	4	1	5	7	1	8	2	1	3	8	—	8
N/60-N/80 ...	7	—	7	2	2	4	6	1	7	6	1	7
N/80-N/100 ...	—	—	—	3	2	5	3	1	4	10	2	12
N/100 and less	—	4	4	—	2	2	3	—	3	1	—	1
Total ...	15	5	20	20	8	28	19	4	23	29	4	33

dangerously low alkalinities of N/80, and less, with an increase of the specific gravity of the blood on admission. All the four cases showing very low alkalinities with specific gravities on admission below 1060 were admitted late with uraemia, which ultimately

proved fatal, so none of the mild cases coming in the acute early stages showed very low alkalinities. Of the fairly mild cases, with specific gravities between 1060 and 1063, 25 per cent. showed very low alkalinities, while those with specific gravities of 1064 to 1065 and over 1065, respectively, showed 30.4 and 39.4 per cent. of very low alkalinities. The close relationship between the amount of fluid lost from the body and the decrease of the alkalinity of the blood confirms the opinion expressed in my previous paper with Captain Shorten, that the reduction of the alkalinity of the blood is an important and essential feature of cholera, and one which requires to be adequately dealt with in any complete and trustworthy system of treating this deadly disease.

Table III shows the alkalinity of the blood in relation to the

TABLE III.—Duration of the Disease and the Alkalinity of the Blood.

Alkalinities	To 12 hours				12 to 24 hours				24 to 48 hours				Over 48 hours			
	Cured	Died	Total	Percentage	Cured	Died	Total	Percentage	Cured	Died	Total	Percentage	Cured	Died	Total	Percentage
Under N/45 ...	11	2	13	27.7	4	1	5	19.2	4	—	4	22.2	2	—	2	15.4
N/45-N/60 ...	8	2	10	21.3	5	—	5	19.2	6	1	7	39.0	2	—	2	15.4
N/60-N/80 ...	10	2	12	25.5	4	—	4	15.4	4	—	4	22.2	3	1	4	30.8
N/80-N/100 ...	5	6	11	23.4	7	—	7	26.9	2	—	2	11.1	2	—	2	15.4
N/100 and less ...	—	1	1	2.1	2	3	5	19.2	1	—	1	5.5	1	2	3	23.0
Total ...	34	13	47		22	4	26		17	1	18		10	3	13	

duration of the disease before admission. The most noteworthy point it brings out is the high mortality among patients showing very low alkalinities within twelve hours of the commencement of the disease, no less than seven out of twelve having died, five from

collapse, one from uraemia and one from pneumonia, showing a high initial severity. Cases admitted between twenty-four and forty-eight hours of the onset are nearly always mild, and they showed the least reduction of the alkalinity. Those coming more than forty-eight hours after the onset showed alkalinities of N/60 and under in 70 per cent., and the three fatal cases all died of uraemia, having been admitted with suppression of urine of one to two days' duration.

THE VALUE OF INTRAVENOUS INJECTIONS OF SODIUM BICARBONATE IN THE PREVENTION OF POST-CHOLERAIC URAEMIA

In 1891, Wall recommended for intravenous injection in cholera a solution containing 0.4 per cent. of sodium chloride and 0.2 per cent. of sodium bicarbonate, and many workers in India adopted his formula, although the amount of sodium chloride was dangerously low, for I found that such a solution haemolysed the blood in vitro. Sellards in 1910, in the Philippine Islands, observed that large amounts of alkalis could be tolerated by cholera patients with threatened uraemia without the urine being rendered alkaline, and he greatly reduced the death rate from post-choleraic uraemia by intravenous injections of sodium bicarbonate. Both Sellards in the Philippines, and J. W. D. Megaw, I.M.S., in Calcutta, however, found that once uraemic symptoms had supervened, it was too late, as a rule, to save the patient by alkaline injections. The earlier estimations of the alkalinity of the blood in my cholera cases by Captain Shorten, showing the constancy with which it is reduced and the extreme degree it reaches in post-choleraic uraemia, led me to inject solutions of sodium bicarbonate as a routine method in the early stages of cholera to prevent acidosis, in the following manner. All cases admitted on the first day of the disease, and requiring transfusion, received the hypertonic solution, namely, sodium chloride 120 grains and calcium chloride 4 grains in one pint. If the case were sufficiently severe to necessitate a second injection, the first pint consisted of sodium bicarbonate 160 grains (2 per cent.) and sodium chloride 60 grains, and this was followed by hypertonic solution up to the total amount required as

judged by the specific gravity of the blood, and this sequence was repeated with each subsequent transfusion. In cases admitted on the second or later day, if suppression of urine was present, the above alkaline solution was used at the first as well as at each subsequent injection. The bicarbonate of soda is sterilized in the solid form under pressure in weighed packets of 160 grains each and added to the sterile solution, so as to avoid the chemical changes which would result from boiling a solution of the alkaline salt.

TABLE V.—Amounts of Sodium Bicarbonate injected in relation to the Alkalinity of the Blood on admission.

Alkalinities	Nil			160 grains			320 grains			480-800 grains			960-1440 grains		
	Cured	Died	Total	Cured	Died	Total	Cured	Died	Total	Cured	Died	Total	Cured	Died	Total
Under N/45 ...	9	—	9	5	1	6	1	1	2	2	—	2	—	—	—
N/45-N/60 ...	13	1	14	7	1	8	1	1	2	—	—	—	1	—	1
N/60-N/80 ...	4	—	4	9	—	9	1	1	2	6	—	6	—	3	3
N/80-N/100 ...	4	—	4	4	5	9	3	1	4	1	—	1	2	1	3
N/100 and less ...	—	1	1	—	3	3	3	—	3	1	—	1	—	—	—
Total ...	30	2	32	25	10	35	9	4	13	10	—	10	3	4	7
Collapse ...	—	2	—	—	5	—	—	3	—	—	—	—	—	1	—
Uraemia ...	—	—	—	—	4	—	—	—	—	—	—	—	—	3	—
Others ...	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—

The first column of cases not receiving any alkalies includes all the mild cases requiring either no intravenous saline or only one hypertonic injection. The two cases which died of collapse were admitted with comparatively low specific gravities, probably due to previous anaemia. In the second column the cases received only

160 grains of sodium bicarbonate, namely, one pint of the 2 per cent. solution. No less than 4 out of the 35 died of uraemia, having been admitted 22, 22, 32 hours and 6 days respectively after the commencement of the disease, while in three of the four the specific gravity of the blood was below normal, so only subcutaneous injections could be given. They all occurred soon after I had commenced the alkaline injections, and before it was known what large quantities could safely be injected in post-choleraic uraemia, so it is possible that some of them might have been saved by early and repeated injections of sodium bicarbonate. The alkalinity on admission was found to be N/120 in two, and the extreme degree of N/240 in a third. The fourth case received five intravenous injections, but, unfortunately, sodium bicarbonate was only given once, this being one of the first cases in which it was used.

The third and fourth columns show a marked contrast to the above, for among twenty-three severe cases requiring several transfusions, but in whom from 320 to 800 grains of sodium bicarbonate were injected intravenously, not a single one was lost from uraemia; thus confirming the above opinion that too small amounts were injected in the fatal uraemia cases shown in the second column. The last column shows the worst cases in whom from four to seven intravenous injections were required, and three of them were eventually lost from uraemia in spite of the injections of from 960 to 1,440 grains of sodium bicarbonate intravenously in the course of several days. Two of the three were admitted fifty hours and four days respectively after the onset of the disease with prolonged suppression of urine, having come too late even for the alkaline injections to save them. The details of five cases are given in Table VI, which will serve to illustrate the principles of the treatment adopted for the prevention and control of post-choleraic uraemia. The daily figures of the specific gravity of the blood, blood pressure, alkalinity of the blood, saline injections, grains of sodium bicarbonate injected, and the ounces of urine passed daily, are given for each case.

In my book on cholera I gave a table on similar lines to the above, illustrating the relationship of the specific gravity of the blood and the blood pressure to the secretion of urine, and showing that a high specific gravity, indicating great concentration of the

TABLE VI.—Cases illustrating the use of Alkaline Injections in Cholera.

Duration on admission		DAY IN HOSPITAL							Totals
		1	2	3	4	5	6	7	
CASE 1—57 hours :—									
Specific gravity ...	1063	1051	1050	1054	1052	1050	—	—	—
Blood pressure ...	65mm.	105	115	120	115	107	—	—	—
Alkalinity ...	N/80	—	—	N/80	—	—	N/40	—	—
Pints of saline ...	—	5½	1	—	1	1	1	—	8½
Grains of sodium bicarbonate ...	—	320	160	160	—	160	160	—	960
Urine, ozs. ...	—	?	?	?	30	20	35	—	—
CASE 2 :—									
Specific gravity ...	1064	1056	1063	1064	1060	1062	1058	—	—
Blood pressure ...	75mm.	93	94	90	101	104	101	—	—
Alkalinity ...	N/50	—	N/70	N/60	N/40	N/70	N/50	—	—
Pints of saline ...	—	4	3½	9	4	7	—	—	27½
Grains of sodium bicarbonate ...	—	160	160	320	160	320	—	—	1120
Urine, ozs. ...	—	22	28	28	38	16	43	—	—
CASE 3—36 hours :—									
Specific gravity ...	1067	1062	1057	1061	1055	1061	1057	1057	—
Blood pressure ...	Nil	70	82	78	83	73	85	78	—
Alkalinity ...	N/80	—	—	—	N/40	—	N/40	—	—
Pints of saline ...	—	9	—	4	4	3	2	—	22
Grains of sodium bicarbonate ...	—	160	—	160	160	160	—	160	800
Urine, ozs. ...	—	?	6	20	58	18	16	52	—
CASE 4—22 hours :—									
Specific gravity ...	1067	1057	1062	1063	1058	1055	1057	—	—
Blood pressure ...	Nil	82	90	97	93	112	116	—	—
Alkalinity ...	—	—	N/80	N/52.5	N/65	N/80	N/80	—	—
Pints of saline ...	—	4	2	1	1	4	—	—	13
Grains of sodium bicarbonate ...	—	—	160	160	160	320	—	—	800
Urine, ozs. ...	—	6	14	9	0	20	50	—	—
CASE 5—8 hours :—									
Specific gravity ...	1065	1063	1059	1064	1054	1056	1047	1054	—
Blood pressure ...	Nil	Nil	65	Nil	92	100	100	85	—
Alkalinity ...	—	—	—	N/80	—	N/80	—	N/100	—
Pints of saline ...	—	4½	9½	10	7	—	1	—	32
Grains of sodium bicarbonate ...	—	—	640	800	640	—	160	—	2240
Urine, ozs. ...	—	0	5	0	6	12	16	?	—

blood due to loss of fluid, and low blood pressure were the main factors in reducing the secretion of urine leading to post-choleraic uraemia. These points are also brought out in Table VI, and in addition the number of pints of saline and the amount of sodium bicarbonate in grains given daily are shown, together with the estimations of the alkalinity of the blood. The first four cases ended in recovery, and the last proved fatal. Where a ? is entered in the line showing the amount of urine passed daily, it means that the amount was very small and was passed either in the bed clothes or with the stool, so could not be measured. The following are the most important points brought out by these illustrative cases:—

Case 1 was admitted fifty-seven hours after the commencement of the disease with suppression of urine for thirty-eight hours, while the alkalinity on admission was found to have been reduced to the dangerously low point of N/80. In the first intravenous injection on admission 320 grains of sodium bicarbonate were therefore given, and in the course of the next five days four more doses of 160 grains each were injected subcutaneously, making a total of 960 grains. On the third day in hospital the alkalinity was still N/80, but on the sixth day it rose to N/40. During the first three days only very small quantities of urine were passed, but from the fourth day it began to be passed in fair quantities, and he made a good recovery.

Case 2 was also a very severe one, requiring no less than seven intravenous salines amounting to a total of $27\frac{1}{2}$ pints, in which 1,120 grains of sodium bicarbonate were given. The alkalinity was thus prevented from falling below N/70, and the patient recovered.

Case 3 was admitted with the very high specific gravity of 1067, and with no pulse at the wrist. The alkalinity was N/80, and he received 22 pints of saline, containing 800 grains of sodium bicarbonate, intravenously in the course of six days. During the first two days very little urine was passed, but on the fourth day the alkalinity had risen to N/40, and 58 ounces of urine were passed, and she made a good recovery.

Case 4 was also a very severe one, admitted with a specific gravity of 1067, and pulseless. On the second day the alkalinity was found to be reduced to N/80, and the urine was scanty, so

injections of sodium bicarbonate were begun. On the third and fourth days there was complete suppression of urine, but after 800 grains of sodium bicarbonate had been injected intravenously, in the course of four days the secretion of urine became re-established, and the patient made a good recovery from a most dangerous condition.

Case 5 was a most remarkable one, requiring no less than ten intravenous injections, containing 2,240 grains of sodium bicarbonate, in the course of six days. Yet the alkalinity could not be raised above N/80, while it fell to N/100 on the seventh day, and the urinary secretion, which had recommenced on the fifth day, after almost complete suppression for four days, again declined, the blood pressure fell, and the patient died of exhaustion, his life having undoubtedly been greatly prolonged by the alkaline injections, although his strength gave out in the end.

The above cases will suffice to illustrate the great value of the sodium bicarbonate injections in preventing the alkalinity falling to the very low point of N/100 or less, which nearly always ends in fatal uraemia. This method of dealing with the decreased alkalinity of the blood, which has now been shown to be such a constant and important feature of severe cholera, has been used throughout 1915, and the result on the death rate from uraemia is shown in Table VII.

TABLE VII.—Yearly death rates from Uraemia.

Year	Total cases	Uraemia deaths	Percentage	
1912	170	24	14.1	11.1 Without alkalies
1913	200	17	8.5	
1914	222	25	11.2	
1915	225	6	2.7	2.7 With alkalies

It will be seen from Table VII that the addition of alkalies intravenously in 1915 has led to a reduction of the death rate from

uraemia from an average of 11.1 among 592 cases from 1912 to 1914, to one of only 2.7 among 225 cases in 1915, or a decrease of 75.7 per cent. in the mortality from this cause. Moreover, of the six patients lost from uraemia during 1915, three were admitted two or more days after the onset of the attack of cholera with prolonged suppression of urine, so were brought too late for even the alkaline injections to save them.

CONCLUSION

The extensive series of estimations of the alkalinity of the blood in cholera cases dealt with in this paper allows me to go much further than in my first paper with Captain Shorten, in which I came to the conclusion that it was advisable to give intravenous injections of alkalies in cases showing deficient urinary secretion without waiting for uraemic symptoms to develop. Now that my observations have shown that the alkalinity of the blood is nearly always very much reduced in severe cases of cholera, and that the early and repeated administration of sodium bicarbonate intravenously has lowered the mortality from uraemia to one-fourth of its former rate, it is now clear that the rule I have adopted of giving alkalies intravenously in all cases of cholera with each saline injection after the first, as well as with the first injection in late admissions with deficient urinary secretion, is a sound one which should never be neglected.

NOTE ON THE TECHNIQUE OF THE ESTIMATIONS OF THE ALKALINITY OF THE BLOOD

BY

SATIS CHANDRA BANERJEE, L.M.S.

The alkalinity of a solution of chemical substance depends on the presence of free OH ions, and it can actually be measured by methods of Physical Chemistry. But the alkalinity of blood, containing organic and inorganic substances, as well as blood corpuscles which have a different composition from the plasma in which they float, cannot be ascertained by simply measuring the presence of free OH ions.

If alkalinity be measured by the free OH ions, then blood is practically a neutral fluid. But if we take the power of combining with acid as a measure for alkalinity, the blood is distinctly alkaline.

Blood has the power of combining with both acid and alkali, owing to the presence of acid phosphates in combination with proteins as well as inorganic salts Na_2HPO_4 , NaHCO_3 , NaH_2PO_4 , etc., and the measure of such power is dependent on the indicator used; for example, blood serum is acid to phenolphthalin, but it is alkaline to litmus. As its acid-combining power is greater than that for alkali, titrimetrically it must be called an alkaline fluid.

Although the titration method does not give us the true neutral point, and, therefore, the degree of alkalinity or acidity, yet it gives a measure of the amount of acid or alkali which can be added to the fluid without raising the OH ion concentration or H ion concentration above certain low limiting values (Rogers and Shorten).

The peculiar content of blood, in proteins, in carbonates and phosphates of alkali metals, increases the possibility of the amount of acid or alkali which can be set free in the body without causing OH or H ionic concentration to rise so high as to disturb or prevent the metabolic processes in the protoplasm, which are necessary for life (Moore and Wilson).

Again when the serum alone is used for estimation, the alkaline principles of the clot are excluded, and if laked blood is used some uncertain chemical processes are taken into account.

Healthy blood serum contains fairly constant alkaline substances whose alkalinity or power of combining with acid can be easily ascertained by the method described below. From variation in this acid-combining power of blood, we can have an idea of the presence of profound changes in its chemical constitution as is found in cholera, diabetic kala-azar coma, etc., and prompt measures can be taken to bring the alkalinity to, or near, the normal level.

METHOD

The alkalinity of blood was determined by Wright's method, slightly modified. Capsules in which blood is collected are prepared according to directions given by Dr. Wright in his book 'Technique of Teat and Capillary Tube.' The curved end of the capsule should be brought so close as to admit the whole into the bucket of the centrifugal machine. The capsules and pipettes are all carefully neutralised (as in the process of preparation they become alkaline) by washing with weak sulphuric acid, then with tap water, and lastly with distilled water. The two ends of the capsule are broken and to one end a rubber tube is attached, and the washing fluid is sucked in and out several times until the washings with distilled water are neutral (as tested with litmus paper).

After washing, the capsules are completely dried by blowing air through them. Special care should be taken so that no drop of water remains inside.

A capillary pipette with a rubber tube, or Wright's throttle pipette with a rubber teat, can be used. A mark is made on the pipette with paraffin pencil, about 1 or 2 cm. from its end, to mark the unit volume of serum or acid solution.

Blood is collected from the tip of a finger, which should be cleaned beforehand with ether, and a certain time allowed for its complete evaporation. A large puncture is made, and the capsule filled with blood in the usual way, and the ends of the capsule are

sealed by pushing them through melted sealing wax. These should never be sealed by heating.

The capsules are kept in the vertical position, and sufficient time allowed for the serum to separate by centrifugalisation, so that it may be obtained quite free from red corpuscles.

Stock solutions of sulphuric acid of the following strengths are prepared, viz.:—N/25, N/30, N/35, N/40, N/45, N/50, N/55, N/60, N/65, N/70, N/80. If further dilutions are required, they can be prepared at the time of working, small quantities of different solutions being placed in separate glass cups or staining blocks (provided with covers so that the strengths of the solutions are not altered by evaporation).

Place in position a clean slide and a few sensitised red litmus papers (prepared according to the direction of Dr Wright).

Break the capsule containing blood by making a nick with a file a little higher than the level of the blood. Insert the end of pipette and suck or draw in unit volume of serum, then draw in a bubble of air, and again draw in unit volume of any acid solution with which it is to be titrated. Eject both of these on the glass slide. Then mix them by drawing up and ejecting four or five times—then again draw a small part of this mixture and eject it on to the surface of the litmus paper. If a blue colour is not produced, repeat the process with a weaker solution until one is found which gives just a faint blue colour. The alkalinity is between this and the one above it, giving no blue colour, and so is the mean of the two.

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IMMUNITY TO YELLOW FEVER *

BY

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Whether the immunity produced by an attack of yellow fever is permanent or temporary is a disputed point among modern epidemiologists. Naturally a disease produced by micro-organisms, and which recovers spontaneously, must produce immunity, local or general, temporary or permanent, else one would not recover. That it is permanent has been, and is now, the opinion of American epidemiologists, and of the older generation of French and English writers who added so much to our knowledge of this disease by their observations in the West Indies and in Africa.

There was, however, a very common belief among the laity of localities in which yellow fever was endemic, that leaving such focus of endemicity for a sufficient time would restore the susceptibility to the disease. This belief is alluded to by many writers. It was, I think, universally regarded as erroneous by those *not* living in endemic areas, but given more consideration, and sometimes, I think not generally, affirmed by writers who lived in such endemic areas.

On the other hand, a commission of the Pasteur Institute, Marchoux, Salembini and Simond, working in Rio Janeiro in 1903 to 1905, state categorically that the immunity produced by an attack of yellow fever gives temporary immunity only, and that the infection is kept up in endemic centres by recurrent attacks among the indigènes. This view is reiterated by other recent French observers, and so far as recurrent attacks being common, is accepted by Seidelin, Rubert Boyce and others. Indeed, it is fair to say that this view, that recurrent attacks of yellow fever are common, is held by nearly all the writers recently engaged in investigating

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the epidemiology of yellow fever. Seidelin seems to base his belief mainly on the occurrence of sickness in men who have had yellow fever before, which attacks he pronounces to be also yellow fever on account of finding in the blood of these patients the organism he believes to be the cause of yellow fever.

When the statement above made was first quoted by Marchoux and Simond, no evidence therefor was, I think, given. A large amount is given, however, by Simond and his collaborators in the report on the epidemic at Martinique in 1908 and 1909. This report is far stronger in its implication of recurrent attacks than it is in assertions of definite recurrences in individual cases, and it is a strong report. Without criticizing it—this would require greater knowledge of the nosology and epidemiology of Martinique than I possess—it is so at variance with what I have seen in the United States, in Cuba and in Panama, that it seems advisable to give such reasons as I have for the opposite view; especially as one would suppose that in the United States, where greater intervals occur between the epidemics of yellow fever, which have also been apparently more extensive and more severe than those in Martinique, one would be more apt to find recurrent attacks than in that island.

Speaking of the immunity given by an attack of yellow fever being permanent—generally permanent only is meant. Absolute immunity given by one attack is not predicated of any disease.

Obviously the natural method of testing this question is by observation of the exposure of men who have had yellow fever to the infection of that disease, and determining if they contract it again. The existence of the infection and degree of exposure would be judged of by the proportion of cases contracted by those who had *not* had yellow fever similarly exposed to infection-controls. If a negative result is reported, the observation would be convincing in proportion to the number of supposedly immune men thus exposed; the intervals from their last attacks; the degree of exposure, and the certainty that secondary attacks did not occur among men.

Such observations are not rare, for instance at the end of 1879 there must have been a very small proportion of the population of New Orleans, Mobile, and the coast towns between them who had not suffered an attack of yellow fever in that or in previous years.

They were free from yellow fever until 1897. In that year and 1898 there were widespread epidemics, yet recurrent attacks were reported in extremely few cases in these towns in 1897 and 1898—eighteen years later. Certainly no considerable number of well-marked cases could have occurred in those years. The same is true of many other epidemics and many other towns in the United States. We can readily present, then, a large number of people having had one attack of yellow fever exposed, after sufficiently long intervals, to infections very prevalent among those who had not had yellow fever with a report of no, or extremely few, cases of yellow fever among them.

Ought not this to be convincing of the permanency—the general permanency—of the immunity? Put yourself on the other side. The first three factors in the evidence—the number of the people exposed, the lengths of the intervals and the prevalence of the infection—you will readily grant us; but I fear that a question will be raised on the last condition—the certainty that secondary cases do not occur. The question will be asked: Is it certain that yellow fever did not occur, and quite generally, among these people? It would be held that, on account of believing that one attack gives permanent protection, we would not recognise yellow fever occurring among them.

There is truth in this contention. We of my generation have accepted previous opinion on this matter, and, not having found it contradicted by obvious facts, have not examined into the matter critically. We assumed that one attack gave permanent protection. We would then have been little apt to consider an attack of sickness occurring in one who had previously had yellow fever as yellow fever unless it was either (1) well marked or (2) gave rise to yellow fever in others. The first might not be the case with light—certainly not with ephemeral—attacks, the kind which naturally would occur as secondary attacks. The second we will consider presently.

Even if a secondary attack were clearly yellow fever, one with our belief would, in the absence of good evidence to the contrary, question the diagnosis of the previously reported attack. This it is natural to do, because we know how many cases of other disease are diagnosed as yellow fever during an epidemic. I did this

in the case of an officer in this service who died of yellow fever under my care at Chandeleur, in 1890. He was reported to have had yellow fever in 1878. For the acceptance of a secondary attack it might then require that a *well-marked case* of yellow fever occur *twice* in the same person. Now, even under the doctrine of temporary immunity, this would be rare, as those who hold it also hold that there would be a lowered susceptibility from the first attack, and that if the attacks were not very far apart the second one would be mild—or very mild—and that is reasonable. You can see, then, that we have not been in a proper state of mind to examine this part of the question critically, and, indeed, I have not done so. Even allowing for this, the number of second attacks reported in places in which yellow fever occurs only in epidemics—the places in which we would be most apt to expect them—are exceedingly rare. They are reported, however, and the recognition of such cases is proof that our eyes are not so blinded by our belief that we cannot recognize second attacks of yellow fever if they be plain enough. I have seen three cases of yellow fever in people who were said to have had a previous attack. In two of them the evidence for the first attack seemed to me to be slight; the third I could not enquire into.

When we come to individual cases, the men we knew who had yellow fever at one epidemic and who were exposed to it a second time after a considerable interval—and I have seen many such—the most I can say is that, in my acquaintance, I have not known a second attack *to be reported*. Drs. P. and W. had yellow fever in Memphis, Tenn., in 1879 and 1878, respectively, and were not exposed again until 1897 at Edwards, Miss., after intervals of eighteen and nineteen years. P. was not reported to have yellow fever at Edwards, but he was sick there with a diagnosis of malarial fever which was prevalent, and to which he was subject. W. was not sick at all. Lieutenant G. had yellow fever in Brownsville, Texas, in 1882; no exposure until 1898 at Siboney, sixteen years later. He was sick at, or after leaving, Siboney with a diagnosis of malarial fever which was extremely prevalent. He did have malaria; plasmodia demonstrated, and recurrent attacks for about a year; but that by no means excludes the possibility of yellow fever. Dr. P., of this service, had yellow fever at Chattanooga,

Tenn., in 1878, next exposure was at Panama in 1903, twenty-six years interval. He was, after nearly a year's residence in good health at Panama City, sick at Ancon Hospital in 1904, with what I myself thought was going to develop into a severe attack of yellow fever, until the second day, when the case showed itself to be clearly dengue—I am satisfied that this case *was* dengue, although it was before we knew the diagnostic value of the blood picture; for it was a severe case, and such are well marked. The terminal eruption, too, was typical, yet you see that one believing that secondary cases are common, might find fault with the diagnoses of all the above. I could add to my knowledge, I think, from twenty-five to fifty cases to the above; but they would be more or less similar. Some did not get sick on the second or subsequent exposure, yet that is not convincing. Some people who have never had yellow fever go through an epidemic unscathed. Also light attacks might well escape unnoticed.

The difficulty is that we recognize no sign as pathognomonic for all cases of yellow fever, the mild and ephemeral as well as the severe. It is true that Seidelin claims to have such a sign in his *Paraplasma flavigenum*, and also to have demonstrated it in a secondary (ephemeral) case of yellow fever in himself and in others, some of whom showed no signs of illness, i.e., were 'carriers.' Without in any way pronouncing on the validity of his claims, yet until his primary contention is confirmed—the transference to guinea-pigs is certainly discredited—we cannot accept the existence of his bodies as pathognomonic of yellow fever, and hence as proving a second attack.

The second test, that yellow fever was communicated to others from cases of sickness of men who had previously had yellow fever, is again hard for us to apply.

When you consider how freely men 'protected by a previous attack of yellow fever' have been allowed to move from places virulently infected with that disease into susceptible communities in the United States, and how many hundreds of times this has occurred during epidemics, you would think we should have satisfactory data on this subject, positive or negative. If not immune to yellow fever, some of these men should have contracted it, and developed it in an infectable but not infected place, and

even if not diagnosed, it should have infected mosquitos and spread to others in whom the diagnosis would be easy. An epidemic cannot be hidden.

I know of no evidence that this has occurred, yet the evidence to the contrary has not been scrutinized critically. Since we did not believe that secondary cases of yellow fever occurred—or occurred very rarely—if an outbreak occurred we would be little apt to impute conveyance of yellow fever to any sickness of indeterminate nature occurring among people who were 'protected by a previous attack of yellow fever.' Almost any other hypothesis would be accepted to explain it, or it would be left unexplained. Indeed, in times of epidemics, there are so many ways in which infection can be introduced that an outbreak, of which we are not able to explain the introduction, is not to be wondered at.

To use this test, then, we must depend upon the scrutiny of exposure of susceptible communities to cases of sickness of such 'protected' men who had themselves been exposed to yellow fever, and under such conditions *that other sources of exposure to the community are excluded*. Opportunity for this would rarely occur during an epidemic.

As evidence of attacks of yellow fever do not recur among such 'protected' people, these observations, to be convincing, must be on a large scale, there must be many failures of susceptible communities thus to receive infection. This would be negative testimony, and convincing only in proportion to its mass. I know of no positive observations on this subject. It seems useless to relate the twenty-five or thirty—maybe fifty—negative observations I could give you. They are not convincing.

Positively, however, we have in the passenger traffic of the Plant Steamship Line data of sufficient mass to be worth considering. From 1889 to 1897, inclusive, nine years, there was no bar to the transit on these vessels from Havana to Key West and Tampa of passengers 'protected from yellow fever by a previous attack of ten years' residence in an endemic focus,' and a great many of them came: Cubans on their ten years' residence, and Americans on a certificate of previous attack. There were cigar factories in Key West, Tampa and Jacksonville all manned by Cuban employees. There were generally two vessels per week, part of the

time three vessels. Havana was about six hours from Key West and twenty-four from Tampa. I am unable to give the exact number of these passengers, on account of the destruction of the records of the State Board of Health of Florida by fire. Such reports of this Board as are available to me give the number of such passengers as follows:—

TAMPA BAY QUARANTINE

1891—May 1st to October 31st	2,620
1892—May 1st to October 31st	2,684
1893—May 1st to October 31st	2,449
1894—May 1st to October 31st	3,681
Four years	<u>11,434</u>

KEY WEST

1893—August, September and October	3,134
1894—May 1st to October 31st	7,556
One and one-half years	<u>10,690</u>

In addition we have a Havana record showing that about 3,420 passengers were certified for Tampa in 1895 (2,850 from May to October 1st). At the above rate the entries at Tampa would for nine years be 25,726, call them twenty thousand, to be conservative. For the short time of which we have record Key West had double as many entries as Tampa, and this is in accord with my observation at the time and in 1899. It will be very conservative, then, to put the number of so-called 'immune passengers' at thirty thousand for the nine years—it was more likely fifty thousand or sixty thousand. Many entries, naturally, were the same people going backward and forward between Havana and Florida ports. Now remember that this very considerable number of people, and I have given you minimal figures, came from a city where yellow fever was endemic; that they came in hot weather to towns where *Aedes calopus* (*Stegomyia*) were abundant and active, and where people susceptible to yellow fever were also abundant. If, then, any considerable portion of them after arrival had been infective to *Aedes calopus*

(*Stegomyia*), I cannot but think that there would have been at least one outbreak of yellow fever in Florida during these nine years. There was none.

Were these people so exposed in Havana that any considerable number of them would certainly have contracted yellow fever if they were susceptible to that disease? Let us see. That those who had not had yellow fever were liable—and very liable—to contract that disease from exposure in Havana at this time was evidenced by the history of vessels from that port whose personnel had *not* had yellow fever. They frequently brought cases of yellow fever to our quarantine station. In 1895, at the Dry Tortugas, I had thirteen cases of yellow fever on Havana vessels out of a crew list of less than 450 men. Indeed, the crew list of men exposed to infection in Havana was not over half of 450, as the steamers lay in a safe part of the harbour—Criscona—and allowed only a very few men ashore, and were practically free from fever. It occurred (with one exception) on vessels which had lain on the Havana side of the harbour. Unquestionably, then, those susceptible to yellow fever could contract it in Havana at this time.

Compare these observations:—

(1) Four hundred and fifty people who had not had yellow fever from Havana gave thirteen cases of yellow fever, every one of which should have been infective to *Aedes calopus* (*Stegomyia*).

(2) Thirty thousand people from the same place during a period covering the same time give no evidence of infecting *Aedes calopus* (*Stegomyia*); certainly gave no rise to an outbreak in the susceptible communities to which they moved.

You may consider this *proof*; there may not be enough of it to satisfy you; but the mass of this evidence, negative as it is, is sufficient until the contrary is proven to confirm me in my belief that, for sanitary purposes, the immunity conferred by one attack of yellow fever is permanent; that recurrent attacks infective to *Aedes calopus* (*Stegomyia*) do not occur, and that we are justified in basing our sanitary measures thereon. It should at least prevent your acceptance of the doctrine that the immunity conferred by an attack of yellow fever is quite temporary, and that subsequent attacks infective to *Aedes calopus* (*Stegomyia*) are common, and that sanitary measures based on the contrary opinion are unsafe. This

observation—the passenger traffic of the Plant Line—is also inconsistent with the existence of ‘carriers’ as a common phenomenon capable of infecting *Aedes calopus* (*Stegomyia*)—against which, however, a stronger argument can be made.

I said ‘until the contrary is proven,’ because if it be ever shown that an organism causative of yellow fever occurs in men who have had previous attacks of this disease, and is conveyable from them by *Aedes calopus* (*Stegomyia*) mosquitos to other men producing yellow fever in them, I will count the contrary proven. I well know how much more determinative are the results of experimental than of epidemiological investigation; yet in this disease it was, I believe, the latter that gave the key to the problem, which determined the direction of the experimental investigation which demonstrated the method of conveyance. I do not mean that this is the only means of demonstration. Even if the causative micro-organism is not demonstrated, the frequent recurrence of clinical yellow fever in those who have had one attack, as indicated in the Martinique epidemic, *sufficiently verified* would be proof.

There are other epidemiological investigations which are at least consistent with a doctrine of permanent immunity, such as the disappearance of yellow fever from small and moderate-sized towns in the Tropics which received few susceptible immigrants. I do not mean that this always occurs; but it is by no means rare in America. The great decrease of infection in Havana in 1899, due to the falling off of immigration in the previous years, is also consistent with it. This was shown in the small number of cases of yellow fever in the spring and summer of that year as compared with normal years, although the town was full of Americans, who went everywhere, and with *Aedes calopus* (*Stegomyia*).

As I have said, against the existence of carriers the evidence is stronger than that against secondary attacks; or, rather, the data on which it rests—again negative—is greater in amount.

There is no record of yellow fever being contracted in New Orleans during the years 1884 to 1896, inclusive. During this period, thirteen years, the crews and passengers of many vessels from yellow fever ports were admitted to this city. During the quarantine season—May 1st to October 31st, the only part of the year that we will consider—this personnel was held a short time in

quarantine prior to admission. I can find no records of the number of this personnel, but I find, supplying a few gaps by proportionate estimates, that during this period there were held for yellow fever at the New Orleans quarantine nineteen hundred and ninety-four steam vessels and four hundred and seventeen sailing vessels. We cannot well make the crews of the former less than sixty thousand (59,820), or of the latter less than six thousand (6,250), a conservative estimate of the passengers—from Havana mainly—would put them at six thousand (6,700)—a total of over seventy thousand (72,775); there were more likely ninety thousand.

If carriers were at all common among this class of people, with so large a number of people we would expect to find a considerable number. Now the introduction of any considerable number of people into New Orleans infective to *Aedes calopus* (*Stegomyia*) during the summer time should have been followed by outbreaks of yellow fever. As I have said, there was none. There could have then been no considerable number of people infective to *Aedes calopus* (*Stegomyia*) among them. This evidence, then, must be added to that just adduced as against the existence of carriers—at least among the personnel of vessels from yellow fever ports in sufficient numbers to affect sanitary measures at United States ports.

What has been said of New Orleans applies equally to Galveston, Mobile, Pensacola and Savannah; and adding the number of ships' personnel from yellow fever ports admitted into these cities without causing any outbreak of yellow fever among them, the total must be well over one hundred thousand.

It is fair to say that only a small proportion of this personnel, especially of the crew, would be expected to show carriers under the circumstances in which they would be expected to exist, i.e., among people who had suffered from yellow fever and were constantly exposed to it. Yet there were a large number, even if a small proportion, of such men aboard vessels from Havana, Spaniards, Manilla men, Italians and Americans.

It is to be noted that the term 'carrier' is here used in a sanitary sense only, i.e., a vector, one infective to *Aedes calopus* (*Stegomyia*), one from whom the disease can be conveyed to other people by the natural method.

SUMMARY

Is the immunity conferred by an attack of yellow fever permanent, or are subsequent attacks common?

The first is the view held by observers in countries where yellow fever prevails epidemically. The second has been the belief of many—especially of the laity—in endemic foci. It is now held by many eminent investigators who have worked in endemic foci of yellow fever; by the majority of recent writers, I think.

The evidence for the permanence of this immunity ought to be most abundant in places where yellow fever occurs in epidemics, and much is brought forward—negative from the nature of the case. This evidence would rarely be satisfactory to those holding a contrary view, because the belief of the physicians in such places that this immunity is permanent would render them little apt to recognize secondary attacks unless they were well marked, and they would rarely be well marked.

There are, however, some epidemiological data which—as far as they go—are evidence against the occurrence of secondary cases infective to *Aedes calopus* (*Stegomyia*).

Thus, between the years 1888 and 1898, there entered Florida ports over thirty thousand people certified as 'Protected from yellow fever by previous attack or ten years' residence in an infected focus.' They came during the summer, May 1st to October 31st, from Havana, where yellow fever prevailed during this time, to Key West and Tampa—towns full of *Aedes calopus* (*Stegomyia*) and of people susceptible to yellow fever. The time of passage was about eight hours to Key West, and twenty-four to Tampa. As no yellow fever developed in Florida during this period, there should have been no considerable number of secondary attacks infective to *Aedes calopus* (*Stegomyia*) among these people.

That yellow fever could be readily contracted from Havana by people susceptible to it is shown by the fact that during this time four hundred and fifty people from Havana, not certified as immune to yellow fever, yielded thirteen cases of yellow fever at a quarantine station.

As thirteen cases of yellow fever, any one of which should have been infective to *Aedes calopus* (*Stegomyia*), occurred among

four hundred and fifty men who had not suffered from one attack, it would seem that if recurrent attacks were common, enough cases should have occurred among the thirty thousand to have produced an outbreak in Florida. There was none.

The above is also evidence that yellow fever carriers are not as common as are alleged by some modern observers; as is also the fact that the quarantine stations of the United States have for many years passed in a large number of people—well over a hundred thousand—from yellow fever ports with no evidence of their having infected *Aedes calopus* (*Stegomyia*) in the United States.

EXPERIMENTAL RESEARCHES ON THE BACTERIOLOGY OF LEPROSY

BY

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This is a brief account of the results of experiments in connection with the bacteriology of leprosy, which were begun in 1911. For the benefit of English readers, who may not have been able to follow my previous papers in Italian and German, I may state that they contain fuller descriptions and details, together with coloured plates, drawings and photographs, showing the various conditions which may arise after the inoculation of rabbits with leprosy material.

Two rabbits were inoculated with small pieces of a leprotic nodule from a private patient on August 16th, 1911; the material was grafted into the anterior chamber of the eye. In both animals the implantation was successful, and on the 3rd December, 1911, the cornea was incised, and a microscopical examination having shown numerous acid-fast micro-organisms, four other rabbits were inoculated in the same fashion with small pieces taken from the first two animals; of the four only two gave a positive result, that is the inoculation was followed by the production of corneal and iritic nodules. With material from these animals, three other rabbits were inoculated on February 25th, 1912, this being, therefore, the third transplantation or transmission. Two failed to show any permanent lesions, but the third rabbit developed in the course of three months a small grey nodule at the site of the graft, which was surrounded by several minute grey and pink granules which spread over the iris, in addition to a cloudy area on the cornea in the region of the inoculation. This opacity showed very clearly numerous arterioles, which taking their origin from the outer hazy margin reached the centre of the cloudy oval patch. These lesions gradually disappeared after two months' time, and eventually the only traces

to be detected consisted in deformity of the pupil with adhesions, diminution of the anterior chamber and localized opacity of the cornea.

After these initial experiments many attempts have been made with material from eight lepers and numerous rabbits. The leprotic lesions were successfully transferred from the first to the second and third generation, but by no means with uniform results; still I have come to the conclusion that, without varying the original method of inoculation, one can produce leprous lesions in the eye of rabbits in two out of three experiments, the second passage is usually only achieved in one out of four attempts, whilst the third removal shows a still smaller proportion of success, though even then by dealing with big series it is possible to prove that inoculation has taken place with living bacteria.

Concurrently to these experiments, very numerous and repeated attempts were made to obtain an artificial culture of the bacterium of leprosy on laboratory media, but all efforts, with one single exception, were sterile, though many different media were employed, both aerobically and anaerobically.

Once only, from a rabbit which had been inoculated 128 days previously, I succeeded in isolating an acid-fast micro-organism, identical in its morphology with Hansen's bacillus, and which I consider to be similar to the bacterium cultivated by Kedrowsky from human lepromas, though possibly my strain may differ slightly in some of its characters and properties from the Russian prototype. The culture was obtained on a single tube of egg-yolk-agar which was anaerobically incubated during a month at 37° C. Two minute wrinkled colonies were just visible, the size of pin-heads, with a peculiar pale yellow wrinkled surface; microscopically these colonies were found to consist of acid-fast bacilli, mostly in thickly matted groups similar in disposition to Hansen's bacillus as seen in lepromas; individual rods were beaded and appeared to consist of a row of extremely acid-fast granules, a feature which is also peculiar to the bacillus of leprosy. Later sub-cultures grew much more rapidly and became adapted to aerobic conditions, and could be made to thrive on most of the usual laboratory media. The optimum temperature is 37° C. The resemblance to *B. tuberculosis* is very remarkable, but this culture is easily identified by injection into

guinea-pigs. Old cultures have a tendency to lose their acid-fast properties and show numerous clubbed shapes. Young cultures (10 days) on glycerine-broth show clumps and radiate arrangement which renders them very similar to the streptothrix group.

Injections of emulsions of this bacterium into the anterior eye-chamber of rabbits have been constantly negative; a parallel experience to that obtained by injecting ground-up lepromas in the same fashion. White mice, guinea-pigs, rabbits and two monkeys have been subcutaneously and intra-peritoneally injected with emulsions of this culture, but so far only temporary, localized lesions have been obtained, if at all.

These investigations are being continued, for much remains as yet to be done; still, at the present stage they appear to me to allow the statement to be made, that, under definite experimental conditions, leprosy can be communicated to rabbits, and that a bacterium similar to that obtained by Kedrowsky can be isolated in pure culture from the experimental lesions of the eye of the rabbit.

Similar results have been achieved by Serra, Chirivino, Bayon and others, but in any case the experimenter must expect to have to face numerous incomplete results or negative inoculations and sterile culture tubes. The impatient observer will easily adopt the opinion of those authors who consider that the experimental transmission of leprosy or the artificial cultivation of its bacillus is impossible and has never succeeded.

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groupings. The culture has a tendency to lose their original
properties and show numerous chemical changes. Young cultures
to have an effect on the growth and the metabolic activities
which involve their very nature in the reproductive group.

Location of colonies in the medium and the nature of
the colonies are related to the growth of the organism. A typical
example is that obtained by growing *Staphylococcus aureus* in the
medium. When the growth is in the medium, the colonies are
seen to be numerous and very densely packed with
colonies in the medium, but as the colonies grow, they become
less numerous and the colonies are smaller.

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A SUDANESE MADUROMYCOSIS

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INTRODUCTION

In 1842, Gill, of Madura, in his Dispensary Report, drew attention to a disease of the foot which produced marked deformity and fungoid excrescences discharging an offensive ichorous fluid, while internally it caused destruction of the joints, cartilages and ligaments, the diseased tissue resembling fibro-cartilage.

In 1846, Colebrook, also of Madura, again reported upon this disease, which he said was commonly known in some parts of Southern India as '*Madura Foot*.'

In 1860, Vandyke Carter applied the term '*Mycetoma*' or fungus tumour (*μύκης* a fungus and *οἶδημα* a tumour) to that variety of Madura foot which contained black granules, and one year later included under this name the white or yellow variety of the same complaint with which he had become acquainted.

Thus almost from the commencement of scientific enquiry into the disease, the black and white varieties were grouped together under the same term, and it appears advisable to continue to do this at the present time, although the name now embraces a large and varied series of different pathological conditions.

With this proviso Mycetoma may be defined as:—

'All growths and granulations producing enlargement, deformity and destruction in any part of the body of man, brought about by the invasion of the affected area by certain species of fungi belonging to different genera which give rise to variously coloured and shaped bodies called "grains," which are formed of hyphae with or without chlamydospores, and are found either embedded in the pathological tissue forming the growths and granulations or escaping freely in the discharge from the diseased area.'

The presence of definite grains separates the Mycetomas from the pseudomycetomatous conditions caused by Sporotrichosis, *Framboesia Tropica* and *Angiokeratoma*.

Such a definition, however, covers very wide pathological and aetiological fields, but thanks to the labours of Pinoy, the diseases classified together under the general term 'Mycetoma' may be divided into two very distinct groups, viz.: *The True Mycetomas* and *The Actinomycoses*.

But confusion may arise between the terms '*Mycetoma*' and '*True Mycetoma*,' and, therefore, to prevent this, we suggest the word '*Maduromycosis*' instead of True Mycetoma. The word '*Maduromycosis*' appears to us to be suitable because the disease was known as '*Madura Foot*' long before Carter introduced the term Mycetoma.

The Mycetomas are therefore divided into:—

- A. *The Maduromycoses* are those forms of Mycetoma with grains composed of large segmented mycelial filaments possessing well defined walls, and usually chlamydospores.
- B. *The Actinomycoses* are those forms of Mycetoma with grains composed of very fine non-segmented mycelial filaments, in which usually the walls are not clearly defined from the contents, and in which chlamydospores are absent.

The Maduromycoses may be classified according to the colour of their granules into :—

- I. The Black Maduromycoses.
- II. The White Maduromycoses.

Judging by Balfour's description of a Mycetoma which contained very hard spherical grains of a brick-red colour, in which Archibald found appearances suggestive of an aspergillar infection, there must be a third class, viz.: *The Red Maduromycoses*, but, if so, this division still requires detailed investigation.

We propose in the present paper to restrict our remarks to the Black Maduromycoses of which we have met with examples in the Anglo-Egyptian Sudan, but before so doing we desire to review the work which has already been performed with regard to this subdivision of the Mycetomas.

HISTORICAL

In order to make clear the points which we desire to raise later on, it is convenient to subdivide the history of the Black Maduromycoses according to the continents in which they have been found, i.e., into :—

- (a) The Asian Black Maduromycoses.
- (b) The European Black Maduromycoses.
- (c) The American Black Maduromycoses.
- (d) The African Black Maduromycoses.

(a) *Asian*. Excluding some ancient references discovered by Collas, to which Corre has drawn attention and which will be considered when we discuss that author's writings, the history of the Black Maduromycoses commences in 1845 in India, where Garrison-Surgeon Godfrey, in his Departmental Report of the Public Dispensary at Bellary, described the occurrence of a considerable black deposit, much resembling fragments of coal, in a foot which had been amputated because it was affected by a disease which was commonly known as '*ulcus grave*,' because the ulcers and sinuses produced such a serious condition that amputation became necessary. This disease he had described in the same report for the preceding year, designating it '*morbus tuberculosis pedis*,' because, though he recognised it to be dissimilar from other recorded

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diseases, he looked upon it as a local tubercular affection, and, influenced by this view, he considered the black particles mentioned above to be accidental, and not essential parts of the disease. He also mentions that it was known to the natives as *Ghootloo Mahdee*, from the tubercular irregularities being supposed to resemble eggs.

This first case of Black Maduromycosis occurred in a native aged about 30 years, and had existed four to five years before amputation was performed. The morbid appearances are described as being similar to those fully set forth in his 1844 report, with the addition of there being in this instance one cyst (or excavated tubercle) containing melanotic matter about the size of a small walnut and extending from the plantar to the dorsal aspect of the foot between the metatarsal bones of the great and second toes, which were in part absorbed. The integuments were not involved in this mass, which when recent had an angular and brilliant black appearance much resembling a fragment of coal, and was considered to be an accidental product in this peculiar case.

Carter says that the second volume of the *Indian Annals* (probably dated about 1849) on page 706 contains an account of dark granular or black gritty particles being found among the bones and in the sinuses of a diseased foot, but we have been unable to refer to this work, and are ignorant of the name of the discoverer and of the date and place in which this observation was made. The particles in question were examined microscopically, and were believed to consist entirely of dried blood, a belief which lasted for many years.

It may perhaps be advisable at this point to draw attention to the fact that Ballingall's celebrated observations do not refer to the black but to the yellow variety of Mycetoma, and hence do not enter into this history.

Sub-Assistant Surgeon Bazonji Rustonji (1858), of the Bhoo's Dispensary, in the Province of Kutch, drew attention to the fact that there were two forms of the disease, viz.: one in which there was no granular deposit, but only a substance dark in colour and soft and thick in consistence; while the other showed small, soft, yellowish granules. This is the first occasion, as far as we know, when a differentiation was made between the Melanoid and the Ochroid varieties of the disease, but Rustonji did not recognise the fungal nature of the bodies in question.

Eyre (1860) states that in every foot examined by him there were numerous minute tubercles resembling fish roe, which were found lying beneath the muscles and extending from the bones to beneath the skin, with nodules of the same appearance and often black in colour. This paper deals with the external characters of the disease, its previous history, natural course, morbid anatomy, aetiology (which was doubtfully thought to be somewhat tubercular) and treatment.

In 1860, Vandyke Carter began that series of classical observations upon the Black and Yellow forms of Madura Foot, which he continued until 1874, and during which he firmly established the fungal nature of the disease.

His first paper (1860) was entitled '*On a new and striking form of fungus disease affecting the foot and prevailing endemically in many parts of India.*' In his second publication (1860) he clearly differentiated between the white or ochroid division of the Mycetomas which to-day we call '*Actinomyces*,' and the black or melanoid variety which we now name *Black Maduromycosis*. He demonstrated that the black grains were of true vegetal nature, with a black friable rind composed of clear orange tinted, ovoid or angular cells and beaded fibres closely arranged so as to form a compact structure, and in addition larger vesicular bodies (seemingly comparable to gemmules or sporangia), which he thinks may arise at the extremities of the compressed beaded fibres by gemmation and expansion. The pale reddish-brown central part of the larger sclerotes was composed of slender, pale, flattened and branching fibres arranged in bundles and intermixed with numerous granules and a few large beaded fibres, the septa of which were sometimes absent.

He placed some black particles, taken from a foot, on cotton soil moistened with animal juices and enclosed in a stoppered bottle, which he left unopened for $2\frac{3}{4}$ years, when he found a thin reddish film had appeared. Other black particles sown on rice paste for the same length of time remained unchanged, but on opening the bottle a red mould speedily made its appearance.

With reference to this mould, he says: 'It had not, however, a clear connection with the fungus particles, but seemed to spring up independently of them upon the rice whenever this was exposed to the air.'

This statement is of importance, as he grew a fungus from the white variety which was pink in colour, and produced sporangia resembling those of a species of the genus *Mucor* Micheli 1729, but differing therefrom in the absence of a columella which should have brought it under the genus *Mortierella* Coemans 1863, but Berkeley, who examined the growths from a botanical point of view, classified it under the genus *Chionyphe* Thienmann 1839, calling it *Chionyphe carteri* Berkeley 1862, and defining it as:—‘Hyphasmate ex albo flavorubroque, sporangiis demum coccineis, sporis breviter fusiformibus.’

The genus *Chionyphe*, however, was never recognised by mycologists generally, as its species came under the genera *Mortierella* or *Mucor*, while *Chionyphe carteri* was most undoubtedly a contamination as its connection with the black or white grains was never proved, as we have noted above with regard to the former.

Thus we may conclude that although Carter gave the first proof of the parasitic nature of the grains, he was unable to produce growths by cultivation from either the black or the white varieties.

In 1860, Minas wrote upon *Keereenagoah* of the Foot, as seen in the Punjaub. The term used is a vernacular word signifying worm disease. He states that the characteristic symptom of the complaint is gradual enlargement of the foot, usually starting with a swelling in the sole associated with the presence and constant discharge of small particles, either soft or black and hard, from fistulous openings.

Collas (1861) described Black Maduromycosis as seen in Pondichèry. He recognised the little bodies of blackish or reddish brown colour which in their clearer parts seemed to be formed of small transparent cells, which he could not sufficiently study. He called the disease ‘*Dégénération endémique des os du pied.*’

H. J. Carter (1862) came to the conclusion that the fungus of Black Maduromycosis was nearly allied to *Mucor stolonifer* Ehrenberg, 1818, the spores of which in an amoeboid state he considered entered the body through the sudorific ducts. Berkeley (1862) mentioned the fungus in question; he gave it the name *Chionyphe carteri*, a nomenclature which he subsequently repeated (1865).

In 1867, Moore reported an important early case in which he effected a cure by cutting and scraping away all the diseased tissues, and he agumented this in 1873 by recording two more cases of a similar nature, treated in the same way with a like result.

In 1870, Holmsted, of Hydrabad, Sind, found a thorn of irregular shape and half an inch long in a case of Black Mycetoma, in which it had been embedded for two years. In the same year, Bristowe described and figured the fungus seen in the black particles of a foot from a case of Black Maduromycosis amputated in Cantoor, and demonstrated to the Pathological Society of London by Tilbury Fox. Bristowe's descriptions and figures are excellent, and amply confirm Vandyke Carter's work. Thudichum chemically examined the black pigment of this case, and showed that it was not derived from blood.

Hogg (1872) described a Black Maduromycosis from India, in which he was able to observe the fungal threads and to resolve them into jointed dissepimented cells, some branching out and attaining a considerable length, while others terminated in an enlarged ovoid head. He, however, believed that the fungus was a secondary product, which might greatly aggravate but did not originate the disease, and suggested that it might be introduced at the time of the first accident when the foot was struck against a stone, or by the poultices used as treatment in a later stage.

Vandyke Carter (1874) published his monumental and classical work '*On Mycetoma or the Fungus Disease of India*,' which concluded his long continued labours at this complaint.

Lewis and Cunningham (1875) admitted the fungal nature of the black particles, but not of the yellow granules. They showed that *Chionyphe carteri* had nothing to do with black or yellow grains.

In 1876, Berkeley came to the conclusion that *Chionyphe carteri* had nothing to do with Mycetoma, a point which can be easily judged from the passages quoted above.

Notwithstanding all these researches, a great deal of confusion still existed with regard to the disease, which can be judged by a study of Fox and Farquhar's (1876) report. It was admitted that the black granules were fungal in nature, but it was contended that they were not causal in effect, because all the essential features of Mycetoma were found to be present without any black fungal particles, and because there was not sufficient evidence forthcoming at the time in proof of the vegetal character of the yellow grains, which were believed to be essentially fatty in nature. It was,

however, admitted that Moore's observation showing that the black variety could be cured by excision of all the particles at an early stage of the disease was a strong argument in favour of the parasitic nature of Mycetoma.

Though Carter had found black, yellow or white, and red grains, still the general belief was that these were one and the same process, and, moreover, observers of this period must have seen the Pseudo-Mycetomatous conditions mentioned above, because competent workers appear to have met with cases in which they were unable to find any grains, although the clinical appearances resembled Mycetoma.

Corre (1883) placed in order, completed and revised the notes of researches made by Collas since his publication, already mentioned, in 1861. In these notes, which were published after his death, Collas desired his previous name for the disorder to be altered to '*La Maladie de Ballingall*,' and states that the earliest references to the disease with which he is acquainted can be found in Waring's paper, and in one of the sacred books of the East which he calls '*Vāveda*' (Ushta wunga hrethayum), which appears to us to be the '*Atharvavēda*.' In this latter work, '*Slipatham*,' or elephant foot, is distinguished from '*Padavalmicum*,' which refers to an incurable malady of the foot associated with swelling and the formation of fleshy tumours, from which, about a year after the appearance of the first symptoms, there exudes a peculiar fluid. He also points out that the words *Perikal*, *Anaikal* (Tamil)—this means Cochin leg—*Slipada* (Bengalese), *Hatty-ka-poung* (Dekkan), are applicable to Elephantiasis as well as to Madura Foot, and, therefore, should not be specially applied to the latter, as they really mean the 'leg of an elephant.' In Ballary, he says, the disease was called '*Gootloo Mahdee*,' because the swellings on the foot were thought to be like eggs; while in Rajputana it was called '*Kirinagras*,' or the dwelling house of worms, because the sinuses were considered to be like the cavities often occupied by the larvae of flies. He also says that in 1714 a missionary described under the name of *Fourmilière des vers* a disease of Pondichéry which was incurable, and in which numerous ulcers intercommunicated by means of small canals full of worms, which were peculiar in that if one closed

another opened. This information Collas obtained from Volume II, page 167, of a book published in Paris in 1812, and entitled *Mémoires sur les mœurs et coutumes de l'Inde par un missionnaire*. Collas also points out that in 1806 Heyne saw the brother of a Rajah at Cuddapah in Hyderabad with a foot in a leprosy state, but which was considered to be distinct from leprosy, although it was not known what the nature of the disease might be. Collas thinks that this must have been Mycetoma, and draws attention to Brett's *Sarcomes adipeux*, in which he says it is difficult not to recognise Ballingall's disease.

With reference to the above names, it will be noted that they apply to any form of Mycetoma, and not especially to Black Mycetoma. The name *Ballingall's Disease*, in our opinion, is not applicable to the Black Mycetomas, because, as already indicated, he was not acquainted with the disease.

In 1886, Carter gave up his pink mould, and drew attention to the similarity between the fungus of Actinomycosis and that of Mycetoma.

Kanthack (1893) studied both the Yellow and Black Mycetomas, and came to the conclusion that the former agreed morphologically and structurally with Actinomycosis, but with regard to the black grains his position was curious, for although he found them to consist of an olive-brown, glassy or finely granular material, in which hollow filaments, radially arranged, were embedded, still he regarded these as degeneration changes, and sought to prove that the granules were an organism allied to the Actinomycosis fungus which he had found in the yellow variety. Thus, like Vandyke Carter, he believed both varieties to be fungal in nature and to be caused by the same fungus, but he attempted to show that the fungus of the yellow variety existed in the black, while the former observer believed the reverse to be true. He named the fungus *Oospora indica* Kanthack 1893, and distinguished the two varieties as *O. indica* var. *flava* and *O. indica* var. *nigra*. Unna, however, to whom he sent specimens, did not make this error, but says: 'A whole series of important distinctions separate the two fungi, and there is no question of their identity.'

Boyce and Surveyor (1894), in a most important paper, first definitely proved that the fungi existing in the black

and yellow varieties were quite different, and thus definitely established the two main divisions of Mycetoma, which to-day we call Maduromycosis and Actinomycosis. They showed that the black grains were composed of a large, septate, branching fungus embedded in a brown pigmented ground substance, which was readily bleached by Eau de Javelle. They did not observe spore formation, nor was cultivation attempted.

In the same year, Boccardo also differentiated between the white and the black varieties of the disease.

Chatterjee (1911) observed that grains placed in agar and glucose agar tubes increased in size some seven to eight times in four days, and were surrounded by fine hair-like structures which were composed of delicate branching mycelial threads, which were seen to come from the thick black threads. On potato, the growth was dry and black. In broth, small white colonies composed of radiating threads were found sticking to the walls of the tube. No diffuse growth was seen, nor did any scum form on the surface. Animal experiments were negative.

Mackenzie, in the same year, appeared to obtain similar cultures on agar; at first the growth was white and translucent, with radiations from the centre, later it became greyish yellow, there being a central granule surrounded by a clear zone and an indented margin. After a week the colony became a deep mahogany, and under the microscope exhibited mycelial structures.

Semon (1915) reported a case of Black Maduromycosis which occurred in a native Indian soldier serving in France. He left India about October, 1914, and in January, 1915, he injured one of his feet by the fall of an ammunition box. The patient attributed the disease to this cause, but Semon considers, probably correctly, that he must have been infected before leaving India. A typical Mycetoma developed in about six months, and the pus contained black particles in which a central mass of mycelium obscured entirely by black pigment could be made out, but no proper demonstration of the fungus *in situ* could be made. The foot could not be amputated, but sections were made of some of the tissue which showed marked vascular hypertrophy, polymorphonuclear, plasma and connective tissue cells, but no endo- or periarteritis and no giant cells.

Excellent growths were obtained at 35° C. on agar agar, maltose agar and Raulin's fluid. With great kindness, Semon sent us one of these growths, which is depicted in fig. 33.

The fungus first formed a central black portion with a peripheral zone of white or grey, which in the course of ten days, or less, became black. The cultures soon became pleomorphic, and lost their definite characters. They showed no chlamydospores when examined microscopically. We have been able to grow this fungus, and can confirm Semon's statements.

Treatment was attempted by giving injections of boiled and unboiled cultures in Raulin's medium, but without effect. Remittent fever, with pain and inflammation of the foot followed treatment by iodide of potassium in 20 grain doses three times a day, while X-rays were useless for diagnostic or therapeutic purposes.

At the present time it is customary to consider that there is only one organism causing this Asian variety of Maduromycosis and that this is the same as *Madurella mycetomi* Laveran 1902, the origin of which we propose to discuss below.

(b) *European.* For a long time Maduromycosis was considered to be essentially a tropical disease, but though this in part holds good, still there are an increasing number of cases being reported in Europe.

The first Italian Black Maduromycosis was discovered in 1886 by Bassini in Padua, in a man who had never left Italy, and who had pricked his foot with an iron pitchfork while working in a cattle stall in 1885. The wound healed, but little by little pain and swelling developed in the region of the healed wound, and these symptoms spread so rapidly that in about seven to eight months the patient was unable to walk, and finally consented to amputation in November, 1886, after which a cure was effected. Bassini gave an excellent and well illustrated account of this case in 1888, recognising that the black grains were composed of radially arranged septate hyphae, 4 to 6 microns in diameter, showing irregular swellings and being embedded in a dark brown matrix. He also observed strands of hyphae, not enclosed in grains, also embedded in a brown matrix. Spore formation was not seen, and cultivation was not attempted. Pepere thinks that, without entirely excluding other possibilities, Bassini's parasite may have been an *Aspergillus* or some closely allied fungus.

Köbner (1891) appears to have reported a case of Black Maduromycosis from Italy, which was thought to be due to a *Mucor* or an *Aspergillus*, but we have been unable to refer to this paper, which is mentioned by Kanthack.

In 1906, Paolo Bovo described a Mycetoma of the foot in an old man in Genoa. The disease was characterized by superficial nodules associated with a single ulcer and by invasion of one of the glands in the groin.

The grains, which were black, varied from a millet seed to a pea in size, and were composed of a black feltwork of filaments and spores, both of which were easily observed. Bovo thought that it might be an *Aspergillus*, but no cultures were made, and very curiously the author insists upon the absence of any inflammatory process and that the single ulcer was due to mechanical attrition.

Brumpt examined Bovo's specimens, and came to the conclusion that it was probably a *Madurella*, and hence it is known as *Madurella bovoi* Brumpt 1910. Pepere, however, says that there can be no doubt as to the aspergillar character of the granules, and as the genus *Madurella* has now been more accurately defined by Brault and Pinoy, it is better to leave this fungus unclassified.

In 1906, Busacchi described a Black Maduromycosis having some analogy with that of Bassini, and occurring in a peasant aged 36 at Cremona. He cultivated the parasite, but he has not yet fully reported upon its nature. It grew well on any liquid or solid media, forming a black or yellowish layer. It was a branching fungus, forming chlamydospores, which he does not consider to be an *Aspergillus*, but thinks that it is a *Streptothrix*, by which he does not mean that it is an Actinomycosis, while he mentions the fact that he had met with a similar case in 1896.

From Pepere's article we gather that Schmincke, in 1910, observed a case of Black Mycetoma somewhat analogous to Bassini's, but found in Kissingen.

A new phase in the history of the European Black Maduromycosis is opened by Pepere's (1914) exceedingly able work on a form due to a *Monosporium*.

Prior to this, Tarozzi (1909) had published a paper, in which he described a white Maduromycosis of the foot in a butcher of Ibono, in the Province of Cagliari, in Sardinia. Radaeli in 1911 gave an account of a case of White Maduromycosis

of the foot occurring in a peasant living in Montemurlo, near Florence, in which the causal organism was recognised to belong to Bonorden's genus *Monosporium*, and was eventually called *Monosporium apiospermum* by Saccardo, in 1911. We have been unable to refer to Tarozzi and Radaeli's original papers, but we have read their controversial papers, and there appears to be no doubt that both met with the same parasite causing White Maduromycosis in Italy and Sardinia.

Pepere's Black Maduromycosis occurred in a peasant, aged 33 years, who lived at Domusnovas, in the Province of Cagliari, in Sardinia. This case was most carefully investigated in every way. Cultures were made on various media and photographs taken therefrom, successful inoculations into the anterior chamber of the eye in guinea-pigs were performed, complement fixation was studied, while the mycology of the fungus was most ably investigated.

The causal organism closely resembled *M. apiospermum*, except that it caused a Black and not a White Maduromycosis, and Pepere, correctly in our opinion, considered that this parasite must be different from *M. apiospermum*, and therefore named it *M. sclerotiale* Pepere 1914. He also used the terms *Monosporium sclerotiale* (*seu nigricans*).

When we compared Pepere's photograph of the fungus with Bonorden's drawings of various species of *Monosporium* we were much impressed with the differences, and we were therefore by no means surprised to read in Pepere's paper that Saccardo proposed to separate *Monosporium apiospermum* from Bonorden's genus and to make a new genus *Scedosporium* for it. This new genus would, of course, contain Pepere's species also, but, unfortunately, we are unable to give a definition of this new genus, as we have been unable to obtain Volume XXII of Saccardo's *Sylloge Fungorum*.

(c) *American*. The next type of Maduromycosis comes from America, where Wright (1898) described a case of black Mycetoma which occurred in an Italian woman, aged 26 years, who had resided in Massachusetts for several years. The disease was first noticed some six months before she applied for treatment, and was confined to the base of the second and third toes on the plantar aspect, which area was swollen and contained a small sinus from

which exuded a dirty greyish fluid containing black, hard, irregular granules, like grains of gunpowder, which were composed of ovoid or rounded translucent bodies of varying sizes, closely packed together, and having a homogeneous interior or containing a few refractile granules. Septate branching hyphae sometimes showing dilatations were also seen, while the periphery of the grain was made up of closely set radiating hyphae, showing more or less marked swellings. All these structures were embedded in a brown refringent substance.

On cultivation in broth, puff-balls were produced which eventually filled the fluid. This finally acquired a deep coffee brown colour, while a film was formed on the surface.

In potato infusion it grew as in broth, but formed no surface growth, and in old cultures gave rise to black sclerotia, 1 mm. in diameter, composed of short thick segmented hyphae and spherical or polyhedral cells with black walls.

On potato it formed a dense, spreading velvety pale brown layer with a white periphery, on which small globules of dark coffee coloured fluid were seen, while the medium became dark brown and very moist.

On agar-agar and glucose agar slants it formed a meshwork of greyish filaments, and in old cultures the black sclerotia appeared, while in stabs there were only surface growths. Spore formation does not appear to have been observed in any of the cultures. Some authorities have considered that Wright's organism was a contamination, but a study of his photographs and description has convinced us that this is not so, and we are inclined to believe that he commenced the cultivation of either a *Madurella* or a fungus like the one we shall describe, which may or may not be different from those obtained in other places. It is curious that the case should have occurred in an Italian woman, but the chance of the infection having been acquired in Italy appears to be remote, as she had lived in America for several years.

It is not definitely stated that she had never visited Italy during that period, but Wright was well acquainted with Bassini's researches, and would probably have drawn attention to the possibility of infection during such a visit if it had been known to have taken place.

We therefore, in the absence of full details, must assume that this Italian woman became infected in the United States and not in Italy, and that the Maduromycosis was American and not European in origin.

de la Hoz (1905) published a thesis on the 'Pathogenic Fungi and Mycoses of the American Continent, but did not increase the number of observations with regard to Maduromycosis.

Seheult (1916) described a case of Black Maduromycosis which occurred in Trinidad, in an East Indian immigrant, who had arrived in the colony in 1899, and whose foot was injured, apparently without causing a wound, by the fall of a cocoa pod in 1908, i.e. nine years after his arrival in the colony.

Three years later, and twelve years after his landing, the foot began to swell and sores appeared upon the instep, and eventually a typical Black Maduromycosis developed, which was said by Balfour to resemble a type found in the Anglo-Egyptian Sudan.

Four years after the commencement of the symptoms, and sixteen years after his landing, the foot was amputated.

This is the first case of Black Maduromycosis to be recorded in the West Indies, and is the second, as far as we know, to be published with regard to the American Continent, though it is possible that we have missed records owing to the limited literature in Khartoum.

It appears to us to be of peculiar interest, because with Semon's and Wright's cases it forms a series of greater and greater possible latency.

I. *Semon's Case* occurred in France, in an Indian soldier who had left India about three months prior to the accident and nine months before the typical Mycetoma developed.

II. *Wright's Case* occurred in the United States, in an Italian woman who had left Italy an indefinite number of years previous to the onset of the disease, which was treated in the same year in which it was first noticed, and after only six months' growth.

III. *Seheult's Case* occurred in the West Indies, in an Indian who had left his native country twelve years before the onset of the disease.

These cases have compelled us to seriously consider the question of latency, and we find ourselves unable to say how long this can

last, and therefore it is possible that Cases I and III were originally infected in India, while Case II may have acquired the infection in Italy.

(d) *Africa*. In 1901, Brumpt, Bouffard and Chabaneix wrote an account of a case of Black Mycetoma which they observed at Djibouti. In the following year, the organism found in this case was studied by Laveran, who gave it the name *Streptothrix mycetomi* Laveran 1902. Brumpt also found the same organism in a Maduromycosis in the centre of Somaliland, and also in an amputated foot sent from Madagascar.

Bouffard, in 1905, reported the presence of the same disease in Senegal and in the French Sudan.

In this variety the grains are black or deep brownish red, and always hard, and generally small, from 1 to 2 millimetres in diameter, when single, and not in accumulated masses. The surface is irregular, with projecting points. On clearing with Eau de Javelle the fungal elements can be clearly seen.

Brumpt (1905) formed a new genus, '*Madurella*,' for this fungus, defining it as follows:—

'Mucedine with white thallus, living parasitically in various animal tissues (bone, muscle, connective tissue), possessing during its vegetative life, filaments with a diameter greater than one micron, and even reaching to 8-10 microns. These filaments are septate and branch from time to time, they secrete a brown substance. When old, these filaments form a sclerote, and their walls sometimes become impregnated with a brown pigment. In this sclerote, there are a number of rounded corpuscles from 8-30 microns in diameter (chlamydospores).'

The type species is the organism called *Streptothrix mycetomi* by Laveran in 1902, which therefore becomes *Madurella mycetomi* (Laveran 1902), and which was first cultivated by Brault (1911) in material from Algerian cases.

This form of Mycetoma was reported by Balfour (1911) to be present in the Anglo-Egyptian Sudan.

It is generally assumed that this and the Asian, together with the American type, are one and the same disease, but this still requires proof.

In 1908, Nicolle and Pinoy described a Maduromycosis which they found in Southern Tunisia, near the Oasis of Tozeur, with hard dark brown grains about the size of a pin's head; in which segmented and ramified hyphae about 1 to 4 microns in diameter were seen,

as were rounded bodies arranged in chains and resembling the mycelial spores of a *Trichophyton*, the whole being embedded in a brownish cement substance. Cultures were obtained at 35° C., and the growths were identical on maltose agar, glycerine agar, potato and carrot, and all the media became pigmented black, due to a tyrosinase produced by the fungus, while the colonies which developed in twenty-four hours at 37° C. were white. Microscopically, the growths showed the 'favic nails' so commonly met with in cultures of *A. schoenleini*. The authors looked upon the organism as belonging to the genus *Oospora* Wallroth 1833, with which Vuillemin considers *Achorion schoenleini* Lebert 1845 should be classified. Its name, therefore, became *Oospora tozeuri* (Nicolle and Pinoy 1908).

Inoculation experiments were unsuccessful in the rabbit, the guinea-pig, and the monkey, but two successful infections were obtained in pigeons.

Brumpt, however, considers the fungus to be a *Madurella*, and therefore its name becomes *Madurella tozeuri* (Nicolle and Pinoy 1908).

Brault (1911 and 1912) cultivated the fungi *Madurella mycetomi* and *M. tozeuri*.

The former grew at 20° C. and 37° C. on broth, various agars, potato, carrot and some vegetal liquid media.

In the liquid media the growth appeared as a whitish grey puff-ball, which later became yellowish or brownish, while the medium remained clear and the growth fell to the bottom of the tube.

On solid media it formed a greyish white, duvet-covered* growth, which possessed a central button surrounded by a radiation, and later, when the culture was drier, the medium became coloured.

Glycerine agar was best, as the growth thereon was luxurious, and when old became yellowish in colour while the medium showed a caramel tinge in its entirety.

Glucose glycerine agar produced a growth of the colour of touch-wood. This culture is thrown into black wrinkles, producing an appearance seen on some sea shores.

When the growths of *M. tozeuri* were compared with those of *M. mycetomi* a number of differences were observed.

* Down-covered.—EDS.

The cultures of *M. tozeuri* grew more quickly, were more luxuriant, and were white, resembling powdered flour. Those of *M. mycetomi* were more discrete grey, 'duveteuse,' radiated, and sometimes showed concentric circles and disassociated more easily than the preceding.

Old cultures on glucose agar or on glycerinated glucose agar were quite different in the two species.

On carrot, *M. tozeuri* attained a deeper brownish yellow colour, while in old cultures on this medium it produces spores in a manner resembling an *Oospora*.

Pinoy, in his remarks upon the mycology of these two species, says that Brault's *M. mycetomi* very closely resembles that isolated by Nicolle under the name *Oospora tozeuri*. Its filaments are 2 to 8 microns in diameter and do not possess apparatus for fructification, reproducing by a breaking up of the hyphae of the thallus into articles 5 to 10 microns in length, which divide into two spores. These spores are of the same diameter as the hyphae from which they arise, varying from 2 to 5 microns, while the membrane becomes yellowish with age. In addition, chlamydospores can be observed forming at the end of the filaments more or less like favic nails. The spores of *M. tozeuri* are smaller, but are formed in the same manner.

On Sabouraud's gelatine, *M. mycetomi* gives rise to black sclerotes in the depth of the medium. These are very numerous, measure half to one millimetre in diameter, and are composed of hyphal segments more or less cylindrical. Sometimes the sphere attains a diameter of 10 microns and usually contains only one nucleus, but, though studied for a long time, these sclerotes were never observed to have any higher form of fructification. In *M. tozeuri* it is very rare to see the formation of sclerotes, which takes place on the surface of the medium.

On the bases of the researches on *M. mycetomi* and *M. tozeuri*, Pinoy classifies the genus *Madurella* as follows:—

Genus *Madurella* Brumpt 1905

Fungi: sterile with septate filaments, reproducing by fragmentation of the thallus. The spores are produced secondarily by binary division of the articles formed. They produce Black Mycetomas in man. They grow well at 37°C.

He further differentiates the two species *M. mycetomi* (Laveran 1902) and *M. tozeuri* (Nicolle and Pinoy, 1908) as follows:—

Madurella mycetomi (Laveran 1902)

Mycelium greyish white, when old, yellowish and darkening the media in sugar cultures. Spores varying in dimension from 2 to 5 microns. Sclerotes black and sterile, with a diameter from 5 to 1 millimetre, formed in the depths of the medium in cultures. Can invade the skin, bone, muscles and connective tissue of man, giving rise to black grains which are small, hard, round and more or less warty, and which morphologically resemble the sclerotes formed in the cultures. Up to the present the inoculation into animals is negative. Very widely spread in Africa. Isolated by Brault from a Mycetoma with black grains in Algeria.

Madurella tozeuri (Nicolle and Pinoy 1908)

Mycelium white, becoming yellowish with age and darkening the medium in sugar cultures. Spores generally small, 2 microns or sometimes even 5 microns in diameter. Sclerotes are only rarely produced, and then they appear on the surface of the medium. Occasionally it gives rise to a Mycetoma in man, in which it forms black amorphous grains which are often made up of mycelial rings enclosing some degenerate cellular elements which are impregnated with the pigment of the fungus, and also of small diffuse masses formed solely by the filaments of the fungus which have a yellow membrane. Inoculation into pigeons positive. Isolated by Nicolle from a Mycetoma at Tozeur.

It will be obvious that the form of spore formation described above gives rise to a *Thallospore*, which is defined by Vuillemin as follows:—

‘The Thallospore is a sporiform element which is really only a portion of the thallus secondarily adapted to the purposes of reproduction.’

The various forms of Thallospores are Blastospores, Arthrospores and Chlamydospores, and it is equally obvious that the spores we are considering are Arthrospores, which are defined as:—

‘A thallospore developed by the disarticulation of hyphal elements at first with square ends, which subsequently become rounded off, and have thin walls, which eventually become thickened.’

The classification of the Genus *Madurella* is therefore sufficiently simple. It belongs to Fuckel's Fungal Class of the *Fungi Imperfecti*

founded by him in 1869, and to the sub-class *Hyphales* differentiated by Vuillemin in 1910, and to the order *Thallosporales*, also defined by Vuillemin in 1910.

This order is divided into two sub-orders, viz.:—

- | | | | | |
|---------------------------------|-----|-----|-----|-----------------------|
| A. Reproduction by blastospores | ... | ... | ... | Sub-order 1 |
| | | | | <i>Blastosporales</i> |
| | | | | Vuillemin 1911 |
| B. Reproduction by arthrospores | ... | ... | ... | Sub-order 2 |
| | | | | <i>Arthrospores</i> |
| | | | | Vuillemin 1911 |

The Arthrospores, which are known to be parasitic in man, are contained in the genera which may be differentiated as follows:—

- | | | | |
|---------------------------------------|-----|-----|---------------------|
| A. Yeast-like forms with short hyphae | ... | ... | Genus 1 |
| | | | <i>Mycoderma</i> |
| | | | Persoon 1822 |
| B. Without yeast-like forms:— | | | Genus 2 |
| I. Producing 'Piedra' on hairs | ... | ... | <i>Trichosporum</i> |
| | | | Behrend 1890 |
| II. Producing 'Black Maduromycosis' | ... | | Genus 3 |
| | | | <i>Madurella</i> |
| | | | Brumpt 1905 |
| III. Producing 'White Maduromycosis' | ... | | Genus 4 |
| | | | <i>Indiella</i> |
| | | | Brumpt 1906 |

Genus *Madurella*. A classification of the species of the genus *Madurella* is as follows:—

Genus *Madurella* Brumpt 1905 emendavit Pinoy 1912

SERIES A. Cultivated and named species:—

- | | | | |
|--|-----|-----|-----------------|
| I. Grains (Sclerotia) large, 0.5—1.0 mm. in diameter. In cultures, spores 2-5 microns in diameter; so-called sclerotes common in depths of media. Animal inoculations: negative. Habitat: Africa | ... | ... | <i>mycetomi</i> |
| II. Grains (Sclerotia) small, the largest rather less than a pin's-head in size. In cultures, spores 2 microns, rarely 5 microns, in diameter; so-called sclerotes rare and only formed on the surfaces of media. Animal inoculations: positive. Habitat: Africa | ... | ... | <i>tozeuri</i> |

SERIES B. Named, but not cultivated, and hence only provisionally placed in the genus:—

- | | | | |
|--|-----|-----|--------------|
| III. Grains (Sclerotia) vary from the size of a millet seed to that of a pea. Habitat: Italy | ... | ... | <i>bovoi</i> |
|--|-----|-----|--------------|

As we have already observed above, some investigators consider the *M. bovoi* will prove to be an *Aspergillus*.

It may be noted that Hatch and Childe describe a case which was a combination of Black and White Mycetomas, but which Musgrave and Clegg consider to be an Actinomycosis.

With regard to Carter's Black Maduromycosis, Semon's researches, in our opinion, show that it is more nearly allied to the fungus which we are about to describe than to Brumpt's genus as defined above, while it is not possible to classify Wright's organism.

But the genera *Monosporium* (*Scedosporium*) and *Madurella* by no means include all the fungi known to cause *Black Maduromycosis*, for Bouffard, in 1905, described a case in Djibouti in which there was no suppuration. The grains were very characteristic, being black in colour and elastic, and breaking when crushed, muriform in appearance, smooth and shiny, and varying in size from a pin's head to a No. 0 shot, and composed of a spirally rolled up mass of hyphae. The pigment is purely superficial, except at a kind of hilum, by which the young mycelial filaments pass out and by their growth increase the size of the grain. The periphery of the sclerote is seen to possess some badly defined granulations, which on careful examination are found to be masses of conidia detached from the conidiophores, while more careful search revealed three heads typical of an *Aspergillus*, and therefore Brumpt gave the fungus the name of *Aspergillus bouffardi* Brumpt 1905. So far it has not been cultivated, nor have inoculation experiments into monkeys, dogs, cats and gazelle been successful.

Thus the Black Maduromycoses may be arranged provisionally into the following groups:—

A. African Black Maduromycoses:—

- I. Brumpt's Black Maduromycosis, found in Somaliland, and caused by *Madurella mycetomi* (Laveran 1902).
- II. Nicolle and Pinoy's Black Maduromycosis, caused by *Madurella tozeuri* (Nicolle and Pinoy 1908).
- III. Bouffard's Black Maduromycosis, caused by *Aspergillus bouffardi* Brumpt 1905.

B. European Black Maduromycoses:—

- IV. Bassini's, Köbner's and Schmincke's Black Maduromycosis, of which the classification of the aetiological fungus is unknown.

- V. Bovo's Black Maduromycosis, of which the causal agent has been called *Madurella bovoi* Brumpt 1910, but this must now be accepted with reserve as it has never been cultivated and may not agree with the definition now given for the genus *Madurella*.
- VI. Pepere's Black Maduromycosis, caused by *Monosporium (Scedosporium) sclerotiale* Pepere 1914.
- C. American Black Maduromycoses :—
 - VII. Wright's Black Maduromycosis, of which the systemic position of the causal fungus is unknown.
 - VIII. Seheult's Black Maduromycosis, of which the nature of the causal fungus is unknown.
- D. Asian Black Maduromycosis :—
 - IX. Carter's Black Maduromycosis, with a doubt as to the exact nature of the causal fungus.

In addition, Black Maduromycoses have been found in various parts of the world, but the descriptions being clinical, these forms are better recorded in the geographical section of this paper, as there is no indication as to the nature of the causal agent.

Having thus defined the present day condition of knowledge of the Black Maduromycoses as far as the limited literature at our disposal in Khartoum permits us so to do, we will now briefly consider the geographical, zoological and botanical distribution of the disease.

GEOGRAPHICAL DISTRIBUTION

Our variety of Black Maduromycosis occurred in the Anglo-Egyptian Sudan, where the disease was first described by Balfour in 1904, and the northern part of which is hot and arid. He gives the native name for Mycetoma as *Napt Hindi Nabit*, and states that the black variety is most frequently encountered, and that the foot is the part principally affected, while the inguinal glands are often involved. In 1908, Wenyon noted its presence at Bor, which is hot but not arid, while Balfour's researches in 1911 have already been noted in the historical section. According to our enquiries, the word most commonly used by natives in the Sudan is '*En-Nabt*,' which means '*The Growth*.'

In addition to the Anglo-Egyptian Sudan, the following is a list of African places from which cases of Black Maduromycosis have been reported :—Algeria, Tunisia, Somaliland, Madagascar, Transkei (South Africa), Senegal, and the French Sudan.

In Asia the disease is recorded from the Yemen, various parts of India, Ceylon, and possibly from North Borneo.

In America it has been described in the United States by Wright, and in the West Indies by Seheult.

In Europe it has so far only been found in Italy, and Southern Germany.

This distribution, according to political geography, has but little meaning when the object being studied is a fungus, and for further details we turn to plant geography. According to Drude, climatic and local conditions permit the division of the surface of the world into six zones of vegetation, viz.:—The Northern Glacial Zone, the Northern Cold Winter Zone, the Northern Hot Summer Zone, the Tropical Zone, the Southern Hot Summer Zone, and the Southern Cold Zone.

The Black Maduromycoses occur in the Northern Hot Summer Zone, which includes Spain and Italy, North America, the Sahara, Indo-China, Malay Archipelago, the United States (roughly south of Utah), and Mexico. The general characters of this region are:—Very hot summer temperatures with cold nights and no real winter, but with varying rainfall. It contains very dry climates; it also contains wet areas. The Black Maduromycoses are most commonly met with in the dry parts of this area.

The Tropical Zone, which appears to be the real home of these fungi, is generally humid, but contains arid regions bordering upon the preceding. In this zone comes the Anglo-Egyptian Sudan, in the northern or more arid part of which Black Maduromycoses are common, and the same remarks apply to Somaliland, while West Africa is mostly moist.

It also includes the greater part of India, in which the distribution of Mycetoma, according to Boccardo, is interesting.

This observer states that Major Prain divided India into six Floral Regions, viz.:—*India Deserta*, *India Diluvia*, *India Aquosa*, *India Vera*, *India Sub-Aquosa* and *India Littorea*, while Black Maduromycosis is found in only *India Deserta* and *India Vera*, and is practically almost absent in other regions.

India Deserta includes the Indus Plain Region, i.e. Sind, Rajputana and the Punjab; while *India Vera* includes the Deccan Region, consisting of the dry but not desert triangle between the

Western and Eastern Ghats, with its apex at Tinnevely and its base at the borders of the plain of the Ganges.

The white varieties of *Mycetoma* are also found in this area, but are outnumbered by the Black Maduromycosis, while in *India Deserta* the preponderance of the Black Maduromycoses is even more marked than in *India Vera*.

In Madura and adjoining districts of Tinnevely, Palmcotta and Coimbatore, situate in *India Vera*, *Mycetoma* is very common, and the climate is hot and arid.

The Southern Hot Summer Zone includes South Africa, where the disease has been recorded, but where it is apparently rare.

This is as far as the present state of our knowledge permits us to go with regard to geographical distribution, and more research on this part of the subject is required, but from the above it is obvious that heat and aridity are favourable conditions for the fungi which cause Black Maduromycosis.

BOTANICAL AND ZOOLOGICAL DISTRIBUTION

Unfortunately, we are in complete darkness as to the characters which the fungi causing Black Maduromycosis assume when not living in animals or on artificial culture media.

Even with regard to those forms of Black Maduromycosis due to an *Aspergillus*, we are quite ignorant as to whether this particular fungus lives on soil or on plants.

Having now reviewed the state of knowledge with regard to the distribution of the disease, we will turn to the description of the variety which we have found in the Sudan.

KHARTOUM CASE

We owe the Black Maduromycosis about to be described to the kindness of Dr. Bousfield, Medical Officer of Health of Khartoum and Omdurman, who removed it entire from the sole of the foot of a native boy, where it was found to be lying superficial to the plantar fascia and beneath the skin. No thorn, splinter or foreign body could be discovered in the tissue, although there was a suspicion that it took its origin from such a source.

The boy made a good recovery, and up to the date of writing

(some six months later) there has been no recurrence. There was no ulceration or suppuration, nor were any of the lymphatic glands of the leg enlarged.

MORBID ANATOMY

The pathological anatomy of Black Maduromycosis has been the subject of a fair amount of investigation. Kanthack merely drew attention to the fact that the black masses were always to be found embedded in dense fibrous tissue, while a few pus and granulation cells were to be seen in most cases. In the fibrous wall, yellowish brown or black pigment could be found, while Fuchsin bodies were present in most specimens. Unna's example, obtained from Kanthack, only showed fibrous and some granulation tissue. Boyce and Surveyor drew attention to the presence of small round cells, macrocytes and giant cells surrounding the fungus in cases of Black Maduromycosis. Their microphotographs are, however, mainly devoted to the fungus, while their fig. 22 evidently depicts a very young piece of fungus surrounded by giant cells.

Wright (1898) stated that the nodules consisted of more or less atypical connective tissue, in the cavities of which the granules lay surrounded by polymorphonuclear leucocytes, loose epithelioid cells and cellular detritus. The cavities were lined by either a wall of vascular granulation tissue or by masses of epithelioid and multinucleated giant cells, while these cells closely invested other granules, and outside of this tissue lay lymphoid and plasma cells. He gives four excellent low-power photographs, of which figs. 4, 5, and 6, though older, if examined with a lens, will be seen to agree more or less with Boyce and Surveyor's fig. 22.

Oppenheim's description in 1904 mainly deals with the fungus, but Brumpt's account of the histological changes induced by *Aspergillus bouffardi* covers all the important points, viz.: the polymorphonuclear leucocytes, the lymphocytes, the giant and epithelioid cells, the connective tissue, the cells containing brown pigment and the endarteritis. On Plate XIX, fig. 7, and Plate XX, figs. 1 and 2, he shows appearances resembling those described by Boyce, Surveyor and Wright in a young grain in which the giant cells are situate close to the fungus.

Boccaro, writing in 1909 in general terms for the encapsulated form of both white and black Mycetomas, says:—

‘The fungal hyphae are surrounded by round cells, held together by a delicate network of fine blood vessels, the cells being located in the meshes of a fibrillar transparent reticulated substance. On the inner side of the group of round cells, between them and the central hyphal mass, is a collection of *finely-granulated debris*, and on the outer side, in most preparations, may be seen large nucleated cells, giant cells, and phagocytes.’

This description, which unfortunately is not illustrated, agrees well with our specimen.

Balfour, in 1911, published photomicrographs of Black Maduromycoses believed to be due to *Madurella mycetomi* and to *Aspergillus bouffardi*, but did not describe them.

Babès (1913) gave a well-illustrated account of Indian Black Maduromycosis, in which he observed far less cells than we have noticed in immediate relationship to the fungus, from which the giant cells were separated by fibrous connective tissue. He drew attention to violet and reddish rounded bodies enclosed in cells.

This, as far as we know, concludes the original observations on the pathological anatomy of Black Maduromycosis of modern date.

In our own case, the tumour described in the clinical section was divided longitudinally into two halves, from one of which the grains were extracted for cultural and other purposes, while the other half was reserved for histological examination.

The tumour measured some 18 millimetres long by 8 millimetres broad by 5 millimetres deep, and was firm to the touch. Fig. 1 shows the general appearance of the growth very slightly magnified. It will be observed to be largely composed of fibrous tissue containing black particles—the grains—and some spaces which are formed by the falling out of some of the black granules during preparation. The spaces demonstrate the character of the lacunae occupied by the grains and their surrounding cells.

Fig. 2 should be examined by means of an ordinary reading lens, when its details can be studied. It will be observed that at the lower part of the photograph lies the fungal mass embedded in cellular tissue. The spaces are artefacts produced in making the section, which otherwise is as natural as possible, i.e. is not bleached or softened in any way. The cracks in the black mass are also artefacts. Around the fungus lies a mass of small cells, and on the

upper and left side of the grain are seen some giant cells, which also occur in other parts but are not in such close relationship to the fungus; then comes some fibrous tissue containing a number of cells, blood vessels and lymph spaces, the last mentioned being situate towards the top of the photograph and being markedly dilated. At the very top of the figure, and only partially shown, comes the dense fibrous connective tissue which is continuous with the dense tissue, depicted in fig. 1, which permeates and surrounds the growth. Therefore the main features of the tumour may be summarised as follows:—

1. Fungus
2. Small cells.
3. Giant cells and large cells.
4. Small cells, connective tissue, blood vessels and lymph spaces.
5. Dense connective tissue.

The description of the fungus will be delayed until the section upon the aetiology, and we will commence our more detailed description with the layer of the small cells.

If the reader examines fig. 7, he will see in the upper part of the photograph a dark area which represents the pigmented fungal mass. Just below this he will observe granular tissue and degenerating cells, while lower down polymorphonuclear leucocytes and some mononuclear cells can be distinguished. The cells are not supported by fibrous tissue, but are separated from one another by granular debris. No blood vessels can be observed.

Fig. 5 depicts in greater detail the degeneration of the cells and the masses of granules which are the product of disintegrated cells.

Fig. 3 shows a number of giant cells, some of which contain fragments of fungal origin. Some large mononuclear cells can also be seen with excentrically placed pale staining nuclei and much granular cytoplasm, which may be vacuolated and may contain the phagocytosed remains of the nuclei of the polymorphonuclear cells as portrayed in fig. 8, which shows a normal polymorphonuclear leucocyte and a large phagocyte filled with engulfed nuclei.

The next layer is illustrated by fig. 9, which depicts at the bottom of the photograph small mononuclear cells, many of which possess a fair amount of cytoplasm, which in some instances is

eosinophile. These cells are separated by a variable amount of fine connective tissue, which also supports large lymph spaces and blood vessels. Débris and pigmentary granules can also be seen. A special rare feature of this layer is the presence of mononuclear cells containing one or more eosinophile rounded bodies, which were first observed in this pathological condition by Kanthack, and subsequently by nearly all the other workers on the morbid histology of the Black Maduromycoses, to which they are, however, not confined. Their exact nature is unknown, but they are probably in some way due to the fungus.

On inspecting the upper part of the cellular mass, it will be observed that the white fibrous tissue increases in amount, but is still loose and contains many cells in its meshes, while more externally, and situate at the top of the photograph, is seen the denser and less cellular connective tissue, which is continuous with that separating one fungal mass from another and surrounding the whole tumour. In this connective tissue, cells containing yellowish granules are frequently observed.

There are also many lymph spaces and blood vessels, but the latter at times show signs of endarteritis (fig. 6) or periarteritis (fig. 4), by which means the lumen of the vessel may be considerably diminished or even closed.

It will be observed that the above description agrees in all important points with those given by other authors, though it varies somewhat in details, and we may therefore assume that there is a general similarity as to the histological reaction on the part of the body against the fungi causing the Black Maduromycoses, and, therefore, we will now pass on to consider the nature of the causal fungus which we have obtained from the Khartoum case.

AETIOLOGY

The outstanding feature of the microscopical specimens prepared from this case of Black Maduromycosis is the presence of black granules embedded in the tissues, as depicted in fig. 1. When these black granules are examined by higher powers of the microscope, they are seen to be largely composed of fungal hyphae

embedded in an interhyphal matrix, as is shown in figs. 10, 22, 23 and 24.

The aetiological importance of these black grains and their contained fungus rests upon the fact that they are present in all forms of Black Maduromycosis, and are co-extensive with the disease, while their complete removal, as in the present instance, effects a rapid and complete cure. Animal inoculations with the grains of Black Maduromycosis are, as far as we know, unsuccessful, while the infection of animals by inoculations of cultures has only succeeded with the varieties met with by Pinoy and Pepere.

Therefore the final and convincing proof of the aetiology of the fungus has only been obtained with regard to two varieties, still, for practical purposes, the aetiology of the other known examples also is firmly established.

The various points to be studied with regard to the aetiological factor in the present case may be divided into:—

1. The histology and mycology of the grain.
2. The cultivation of the fungus.
3. The mycology of the cultures.
4. Classification.
5. Animal inoculations.
6. Comparison with the fungi of other Black Maduromycoses.

1. *The Grain.* When a microscopical section, such as that depicted in fig. 22, is examined, it may be noted that it consists of a central or medullary portion, which is light in colour and is surrounded by a thick, dark, radially striated rind or cortex.

On closer examination, the medullary portion is seen (fig. 23) to be composed of segmented, branched, light coloured hyphae, running in various directions, and of roundish, oval, or irregularly shaped thick-walled bodies, which are obviously chlamydospores. Both these structures are embedded in a light sepia coloured homogeneous matrix, which is very hard and cracks when cut with a razor, as is seen in figs. 2 and 22.

The rind or cortex, on examination with the higher powers of a microscope, exhibits fungal hyphae arranged more or less regularly and radially (fig. 10), thus producing the striated appearance shown in fig. 22. Some chlamydospores may also be seen which,

together with the hyphae, are embedded in a hard sepia tinted homogeneous substance.

The pigment in the grain is solely derived from the fungus, as it can be seen in the cultures, and it appears to us probable that the whole matrix is so derived. It can be bleached by Eau de Javelle or by freshly prepared hypobromite of soda, and when so bleached tinges readily with eosine. In the photographs with which this paper is illustrated, we have preferred to show the grain as naturally as possible, and without bleaching.

With regard to the hyphae mentioned above, they may be seen to differ in no material respect from those commonly met with in fungi.

They are septate and branched, while the interseptal segments measure about 2.1 to 2.8 microns in breadth and some 5.6 to 8.0 microns in length. In some instances they appear to be devoid of contents, but, especially in the rind or cortex which is the growing area, they possess eosinophile granular matter. In this position and towards the periphery of the grain, they are often swollen and assume a knob or club-like appearance at the extremities (figs. 20, 22 and 24), which are in close relationship to the leucocytes shown in fig. 22, from which they are sometimes separated by a hyaline eosinophile substance, which may appear as a series of finger-like processes in places where the leucocytes have fallen away from the grain. In the medulla the hyphae may be seen cut transversely, longitudinally or obliquely (fig. 23).

In places these hyphae may be observed to swell considerably, giving rise to larger or smaller thick walled, rounded, oval or irregularly shaped bodies (figs. 12, 13 and 23), which are to be looked upon as intercalary chlamydospores, which apparently become detached from the hyphae when old, and can be seen lying by themselves. The contents of the chlamydospores vary, being sometimes eosine staining granular material, which at other times may be entirely wanting or may have its place taken by a dark sepia tinted substance somewhat resembling the homogeneous matrix, while much more rarely the contents appear in the form of minute clear bodies (fig. 13). The walls of these spores are usually thick, and sometimes very thick (fig. 12), while they may be light in colour or deeply tinted, being then more or less of a sepia colour.

The chlamydospores which we have measured varied between about 7 microns in transverse diameter in the roundish forms to 15.4 by 14.0 microns in the oval varieties.

That these bodies are really chlamydospores may be judged from the photographs, and from the usual definition of such bodies, which is as follows:—

‘An intermediate or terminal spore larger than an ordinary hypha which, without becoming isolated, undergoes a kind of encystment with the formation of a thick, and sometimes coloured, wall, containing cytoplasm loaded with food material.’

The next point to be considered is the mycological position of the grain, and it appears to us the only fungal structure which at all resembles it is a ‘sclerotium,’ which is defined by De Bary as follows:—

‘The name *sclerotium* has been given to certain thick tuber-like bodies, formed on the primary or filamentous mycelium, which proceeds from the germinatory spore; these, which are storehouses of reserve material, become detached from the mycelium when their development is complete and usually remain dormant for a considerable time, and ultimately expend their reserve material in the production of shoots, which develop into sporophores.’

The grains we are considering do not agree well with this definition. They may be storehouses of reserve material, as the matrix may be of this nature, but it appears to us more probable that it is protective in character, because a grain, so far as our experience goes, will not grow unless placed under extremely advantageous food conditions, e.g. being placed in glucose peptone, thus giving no indication that it contains food materials in itself, as is the case with the sclerotium of the ergot of rye.

Again, it is not a part of the mycelium, but is the mycelium itself, and can grow, which a sclerotium is not supposed to do, while its rind is the actively growing part, though in a sclerotium it should be composed of cells with sclerosed cell walls which are dark in colour and have little solid cellular contents.

It appears to us to be probable that it is from the clubs of the rind or cortex that the hyphae of the puff-ball which forms in glucose peptone may develop, though this is a pure supposition on our part, as we were not in possession of sufficient black grains to test this point, nor, as far as we know, has it ever been investigated by any one up to date.

Moreover, we are not acquainted with any literature describing 'chlamydospores' inside a 'sclerotium.'

We therefore conclude that, although the grain may superficially resemble a sclerotium, it differs therefrom in that it is *the mycelium*, and not a detached portion formed in a mycelium; it can grow, its cortex is not composed of protecting cells, and it is doubtful whether it contains much reserve food material, while finally it does not produce sporophores but a mycelium, which only after a time and after much growth forms chlamydospores and other spores.

We therefore believe that the sclerotium is not homologous with the grain, and we consider that this term should be used in a mycological sense, being called '*granum*,' which may be defined as follows:—

'The name "*granum*" has been given to certain differently coloured bodies of varying consistence, size and shape which are composed of hyphae, and sometimes chlamydospores, embedded in a matrix, and which, on germination, give rise to mycelial filaments, and are found in mycetomas.'

Although there is a marked morphological and developmental difference between a 'sclerotium' and a 'granum,' it must be admitted that they both serve the same physiological function in that they are protective and keep alive the growing elements of the mycelium under adverse circumstances.

2. *Cultures.* When a grain is incubated in glucose peptone medium at 30° C. under aërobic conditions in an incubator it sprouts, producing a large number of white hyphae, which form a structure resembling a puff-ball having the remains of the black grain in the centre. These white hyphae grow until they reach the walls of the glass tube and the surface of the medium upon which they form a skin, whilst the fluid itself acquires a dark reddish brown colour, due to the pigment derived from the fungus.

When placed upon maltose agar, under similar conditions, it produced hyphae, which formed a growth which finally obscured all trace of the original grain. This growth, which is depicted in fig. 14, is black in colour, and has a central elevation surrounded by a depression which separates it from a grooved, raised plateau, which has a slight fringe. At first the growth was greyish white, but gradually it became darker and darker, while the medium also increased its own naturally dark tint. To be exact, the colour of the

growth agreed with Ridgway's Standard Colour 'Dusky Drab,' as depicted in his Plate XLV, 9, ORO, K.

From this culture on maltose agar all the sub-cultures were made.

With regard to its biological characters, the fungus grew under aërobic conditions at 22° C., 37° C., and 40° C., as well as at 30° C., but it did not grow at 60° C. nor anaërobically.

The varying characters of its growths at different temperatures, but on the same medium (Sabouraud's maltose agar) and for the same number of days (eleven), are depicted in figs. 15 at 22° C., 17 at 30° C., 18 at 37° C., and 21 at 40° C.

It will be observed that 30° C. is the optimum temperature, and also that the originally black growth has become covered with a greyish 'duvet.'

When grown in a watch glass, by the method described by Chalmers and Marshall, it produced a growth of which fig. 11 is a photograph, taken on the ninth day. The grooves on the plateau are not so marked as when grown in a test tube. A peculiarity of these cultures is a grey fluffy appearance, which forms on the surface of the black growth when exposed freely to the air in watch glasses, Kitasato flasks and uncapped test tubes. In order to demonstrate the fluffy appearance, fig. 16 is reproduced, which is a photograph of the same culture as that represented in fig. 11, but magnified four times.

Fig. 19 shows a similar growth on maltose agar, but produced in the protection of a Kitasato flask. In all these cultures the black pigment was very marked, as could be seen when the deep surface of the growth was examined.

Fig. 34 shows another growth on maltose agar after fifteen days in a capped tube at 30° C. It will be observed that the greyish fluff is nearly absent, although it is present to some extent in the white marking on the upper part of the plateau and in the upper part of the fringe. The central knob is represented by an elevated, bare, crumpled transverse ridge, while the plateau is nearly black, though it shows groovings, while the fringe is reduced to a mere margin in the lower part, though more marked in the upper portion of the photograph, where it looks rather elevated, which was due to the fluffy hyphae.

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On glycerine agar, glucose agar, and agar agar the growths

resemble that produced on maltose agar. On ordinary agar agar, in eighteen days, it formed a growth with a dark centre and a white periphery. Glucose agar growth in eighteen days resembles that of the maltose agar, but there are at first more white hyphae.

On blood serum it produces the appearance depicted in fig. 38; there being no liquefaction of the medium into which the black growth sunk somewhat and subsequently developed a slight duvet.

The development in a gelatine stab at 22° C. was peculiar, in that a growth formed in the stab just below the surface, and spreading upwards and outwards reached the surface, where it produced a white growth placed superficially to a dark feltwork below which the medium became tinged with a reddish brown colour. On this white surface there appeared a number of small hard black bodies (fig. 29), which upon microscopical examination were found to be composed of dark walled Chlamydospores lying crowded together in masses and having a quantity of free pigment between them (fig. 41). Much as these resemble grains in appearance and also in consistence, they are morphologically very different, being merely collections of chlamydospores. There was no growth in the depth of the stab, nor was there any liquefaction of the gelatine.

The growth upon potato at 30° C. is depicted in fig. 28, when it is observed not to be characteristic, but is seen to be puckered, black and fluffy. Later, after eighteen days (fig. 43) it formed a wrinkled black growth with a black central knob. The potato was stained black around the growth, but was not eroded. In infusions of potato it grew well, forming puff-balls, and eventually a skin on the surface in which black masses appeared which were of the same nature as those seen in gelatine. There was no reduction of Fehling's solution by this growth, indicating that no sugar was formed by the fungus, while tincture of iodine produced the usual starch reaction.

On carrot it grew out into a dark mass covered by a greyish fluff (fig. 25). Cheese and lard were tried, and found to be unsuitable for cultivation purposes, as the fungus failed to grow, although it apparently did not die.

In litmus milk it formed a superficial white skin which was pigmented on its deeper parts, but it did not give rise to acid nor

did it form a coagulum, though it cleared the solution, which took on a port wine tint, while it produced a flaky precipitate which fell to the bottom of the tube.

3. *Mycology*. All growths show septate branched hyphae of varying diameter, from 2·8 to 1·4 microns, but in very old cultures (when all the hyphae are dark) they may measure 4·2 microns (figs. 26; 27, 30 and 31). These hyphae are pale when young (figs. 27 and 31), but are dark coloured when older (fig. 30), taking on a greenish black tinge. The length of an interseptal segment varies very considerably, and perhaps 12·6 microns may be considered as an average, but much less than this have been observed, while segments reaching 28 microns in length have been noted in the dark coloured hyphae. In most of the older cultures, clear or dark coloured thick walled chlamydospores ($14 \times 11\cdot3$ microns) may be observed, but they are especially marked in the black masses present on the surface growths on potato infusion and nutrient gelatine (figs. 29 and 41).

The surface growth on old potato infusions, however, produced another feature, viz., that depicted in figs. 26, 27, 30 and 31, which, if examined, are seen to show a spore formation of a different nature to that of a chlamydospore. These spores are seen to be produced at the ends of hyphae or on stalks which look like sporophores, or to be situate pleurogenously, with or without short stalks, on the main hyphae, vide figs. 27 and 31. The same features, but not so marked, are to be seen in fig. 42, which is taken from an old maltose agar growth.

The spores are noted not to be rounded, as is the case with conidia, but to be truncated by the septa which cut them off from the hyphae, and thus they are not round but oval.

The spore-bearing hyphae are generally branched, vide fig. 31, and often the segment below the spore produces one (fig. 26) or, more rarely, two lateral projections, which make an appearance as though there were three spores, i.e. one central and two lateral.

Another remarkable feature of these spores is their persistence in adhering to the hyphae. Whereas in teased preparations from fungal growths with spores it is customary to find many detached spores lying in the field of vision, it is rare in the present case to

see a detached spore, even when the preparation has been roughly handled on purpose. The spores are unicellular and pale when young.

Black pigment particles may be observed lying between the hyphae.

No sign of any sexual reproduction was observed in our preparations.

4. *Classification.* It is obvious that as the fungus in question is a parasite with filaments clearly septate, reproducing by spores and obvious sexual cells, it belongs to the *Eumycetes* of Schröter, and not to the *Phycomycetes* of De Bary. As the spores are not contained in asci or basidia, it is equally manifest that it must be placed in Fuckel's class called *Fungi Imperfecti*.

As no accessory fructifications can be seen, and as reproduction appears to be by means of spores situate on hyphae, it must be placed in Vuillemin's sub-class *Hyphales*. The further classification depends on the view taken of the spores depicted in figs. 26, 27, 30 and 31.

As they do not appear to be capable of forming new spores or hyphae while still attached to the parent mycelium, they would be classed as conidia, of which there are two main types, viz., the Aleuriospore and the Conidium Verum.

The *Aleuriospore* is a conidium which is characterised by being truncated by the septum which separates it from its parent hypha, to which it is so closely united that it is only set free by destruction of that structure.

The *Conidium Verum* is not truncated, but, on the contrary, is constricted, thus producing a rounded appearance, and is attached to its parent hypha by only a restricted area of its rounded periphery, from which, as a rule, it is easily detached.

The two main features of the Aleuriospore, viz., its broad attachment to the parent hypha and its persistent adherence thereto, are well exhibited in figs. 26, 27, 30 and 31, which are teased preparations.

We, therefore, conclude that these spores are *Aleuriospores*, and with this deduction we are now in a position to proceed with an analysis of Vuillemin's classification of the *Hyphales*.

As the fungus under consideration does not possess a mycelium

composed of bacilliform, but, on the contrary, of broad septate hyphae, it does not belong to the order *Microsiphonales*, and as its method of reproduction is by means of *Conidia*, it belongs to Vuillemin's order of the *Conidiosporales*, which is divided into five sub-orders as follows :—

A.	Conidium imperfect being in the form of an Aleuriospore	Sub-order 1 <i>Aleuriosporineae</i> Vuillemin 1911
B.	Conidium perfect :—						Sub-order 2
	I. True conidiophores absent	<i>Sporotrichineae</i> Vuillemin 1910
	II. True conidiophores present :—						Sub-order 3
	a. Conidia borne on sporophores	<i>Sporophorineae</i> Vuillemin 1910
	b. Conidia borne on phialides :—						Sub-order 4
	1. Prophialides absent	<i>Phialidineae</i> Vuillemin 1910
	2. Prophialides present	Sub-order 5 <i>Prophialidineae</i> Vuillemin 1910

From this table it is apparent that the fungus described above belongs to sub-order 1, *Aleuriosporineae*, which is classified by Vuillemin as follows :—

A.	Conidiophores absent	Family 1 <i>Aleurismaceae</i> Vuillemin 1911
B.	Conidiophores present	Family 2 <i>Monotosporaceae</i> Vuillemin 1911

Figs. 26, 27, 30 and 31 show that true Conidiophores are absent, and that the Aleuriospores are borne either acrogenously or pleurogenously on hyphae, from which they are separated by means of a septum which truncates the spore, and therefore does not produce the constriction associated with the separation of a Conidium from a Conidiophore.

The genera of the family *Aleurismaceae* may be recognised by the following table :—

A.	Hyphae pale :—						Genus 1
	I. Hyphae very short, with sporogenous apparatus but little distinct from the mycelium	<i>Myceliophthora</i> Costantin 1894

II. Hyphae elongate, with sporogenous apparatus distinct from the mycelium. Fertile hyphae branched :—

- | | |
|--|---|
| a. Aleuriospores smooth, small and acro-pleurogenous :— | Genus 2 |
| 1. Aleuriospores coloured | <i>Aleurisma</i>
Link 1809 |
| 2. Aleuriospores pale | Genus 3
<i>Corethrospis</i>
Corda 1839 |
| b. Aleuriospores spiny, large and acro-
genous :— | Genus 4
<i>Mycogone</i>
Link 1809 |
| 1. Aleuriospores appendiculate | |
| 2. Aleuriospores not appendiculate | Genus 5
<i>Sepedonium</i>
Link 1809 |
| B. Hyphae dark :— | |
| a. Aleuriospores small (generally 6×4 , rarely 11×5 , microns), become dark, situate acro-pleurogenously on dark or light coloured hyphae | Genus 6
<i>Glenospora</i>
Berkeley and
Curtis 1876 |
| b. Aleuriospores large (11-14 microns), remain hyaline, situate acrogenously on hyaline hyphae at the base of sterile dark hyphae . | Genus 7
<i>Botryotrichum</i>
Saccardo and
Marchal 1885 |

It will be observed that the fungus under consideration belongs to the section with dark hyphae, and is closely related to the genus *Glenospora* Berkeley 1876, while it is easily differentiated from *Botryotrichum* Saccardo and Marchal 1885 by the size and arrangement of its Aleuriospores.

We therefore consider that in all probability the fungus which we are considering should, at all events, *provisionally*, be placed in the genus *Glenospora*, because its Aleuriospores are small and are situate acro-pleurogenously on dark or light coloured hyphae.

If the older method of classification is adopted it still belongs to the *Fungi Imperfecti*; ORDER Moniliales (*Hyphomycetaceae* Martius 1817), because its hyphae are more or less developed and cobwebby, or more or less compact, and never enclosed in a pycnidium, and typically superficial; FAMILY Dematiaceae Fries 1832, because its hyphae are dark or black, cobwebby, loose, usually rigid, conidia typically dark and concolorous, but sometimes the hyphae are dark and conidia clear or vice versa. As the hyphae are manifest and distinct from the conidia it comes under the *Macronemeae*, and as its conidia are subhyaline or dark, not in chains but inserted irregularly, while in its growths it tends to form a crust, it again comes down to the genus *Glenospora*.

It also agrees well with Lindau's definition of the genus as given in Engler and Prantl's *Pflanzenfamilien*, which reads as follows:—

‘Hyphen und Conidienträger eine schwarze kruste bildend. Conidienträger septiert verzweigt. Conidien endständig und seitenständig meist einzeln, lange anhängend, kugelig, ziemlich gross, grünschwarz.’

It does not quite agree so well with Saccardo's definition, which is:—

‘Hyphae biogenae in crustam atram intextae, variae ramosae septatae. Conidia ramulus diu haerentis, globosa majuscula, levia.’

but this definition is only written to include *G. curtisii* and *G. ramorum*. As we have been unable to obtain any specimens of other species of *Glenospora* with which to compare the fungus which we are considering, we have been compelled to trust to descriptions and illustrations, which are often misleading, and therefore we only *provisionally* classify it as a *Glenospora*.

We now come to the consideration of the species, and we find that Saccardo, in 1886, in Volume IV of his ‘*Sylloge Fungorum*,’ only lists two species, viz.: *G. curtisii* Berkeley and Desmond, which is apparently the type, and *G. ramorum* (Schweinitz 1822), while Lindau, writing in 1900, stated that there were four known species, but the literature available in Khartoum does not indicate what these other two species may be, on the other hand, we are well acquainted with the literature pertaining to the human parasite *Glenospora graphii* (Siebenmann 1889), found in cases of Otomycosis and Keratomycosis.

There is one difference which has impressed us considerably, and this is the variation in the dimensions of the spores, thus Saccardo gives the measurements of the Aleuriospores of *G. curtisii* as being 10 to 12 microns in diameter, while Landrieu gives those of *G. graphii* as 6 to 6.7 × 4 to 5 microns, and only exceptionally 11 × 5 microns, so that the average transverse diameter would be about 4 to 5 microns.

The measurements of the spores in our specimens are about 4 to 5 × 3 to 4 microns, which gives a transverse diameter of about 3 to 4 microns.

We also observed that Lindau uses the term ‘ziemlich gross’ for the spores of the genus.

The Aleuriospores of our specimens are smaller than those of

G. graphii, but, apart from this, there appears to be very little morphological difference between the two fungi. We have inoculated our fungus into the anterior chamber of the eye in rabbits with negative results, as has Landrieu with *G. graphii*. We have never seen any illustrations of cultures of *G. graphii*, but the only differences which we can find are that *G. graphii* only slightly modifies milk (in what way the milk is changed is not stated), and the colonies remain white or grey much longer on serum than on other media, while with our fungus milk is profoundly altered, as described above, and a black growth is quickly formed on serum.

The only important difference is that in man *G. graphii* produces only Otomycosis and Keratomycosis, while our fungus produces a Mycetoma; we therefore conclude that they are different species, and therefore name our fungus *Glenospora khartoumensis* Chalmers and Archibald 1916.

It appears to us therefore to be possible to divide the species of *Glenospora* into two groups, as follows:—

- A. Aleuriospores large, usually measuring ten or more microns in diameter:—
 - Parasitic on Plants { 1. *curtisii*
2. *ramorum*
- B. Aleuriospores small, usually measuring five or less microns in diameter:—
 - I. Parasitic in Man, causing Otomycosis and Keratomycosis 3. *graphii*
 - II. Parasitic in Man, causing Black Maduro-mycosis 4. *khartoumensis*

5. *Animal Inoculations.* It may briefly be stated that experiments with the grains or with cultures in monkeys, rabbits and pigeons intraperitoneally, subcutaneously and deep into the tissues in various parts of the body, associated with or without the introduction of a thorn, and into the anterior chamber of the eye, have one and all proved to be complete failures, the fungus in all cases disappearing without producing any effect. The experiments with cultures were repeated several times, always with the same result.

We suspect that even man must be fairly resistant to the infection, because many native people must be infected through cuts and scratches, after which the fungus must be completely destroyed, while it must be relatively rare for a Mycetoma to develop,

otherwise there would be far more cases of the disease that there are, especially in a country like this, where thorns grow on many plants and where some native tribes go about naked.

Comparisons. It will be sufficiently obvious that *G. khartoumensis* is very separable from the various species belonging to the genus *Madurella* Brumpt 1905, emendavit Pinoy 1912, because the latter belongs to the Hyphal order *Thallosporales* Vuillemin 1910, while the former comes under the Hyphal order *Conidiosporales* Vuillemin 1910.

Therefore the Khartoum Black Maduromycosis can be readily differentiated from :—

1. Brumpt's Black Maduromycosis;
2. Nicolle and Pinoy's Black Maduromycosis;

but it will also be obvious that if the three fungi had not been cultivated then differentiation would have been difficult.

With regard to Pepere's Maduromycosis, we note that *G. khartoumensis* belongs to the sub-order *Aleuriosporineae*, while Pepere's fungus *Monosporium sclerotiale* or *Scedosporium sclerotiale* belongs to sub-order *Sporophorineae*, as set forth above, because its spores are true conidia borne on true conidiophores which are of the type of a sporophore (see Chalmers and Archibald, 'The Fungi Imperfecti in Tropical Medicine'), and therefore the distinction between the two types can be made, but with more difficulty than in the preceding cases.

From Bouffard's Black Maduromycosis it can be separated by the absence of aspergillar heads in the microscopical sections.

It cannot be distinguished at present from Bassini's Maduromycosis, as there is insufficient knowledge with regard to this form.

With regard to Wright's Black Maduromycosis, in fig. 12 of Plate XL, he illustrates by a photograph a culture on potato obtained from the black grains, which is altogether different from the photograph of *G. khartoumensis* as shown in fig. 28, Plate III, of the present paper, so that it seems probable that his Maduromycosis is different from the Sudan variety.

We now come to a much more difficult differentiation, viz., that from Carter's Black Maudromycosis, which would have been impossible without Semon's most valuable and brilliant researches of last year.

It is not our intention to enter into a detailed or mycological description of the cultures which, by the kindness of Semon, we have been able to make from his original growth, as he would have done so himself if not employed on active military service for his country. We merely desire to invite the reader's attention to the cultural differences depicted in figs. 33 and 34, figs. 35 and 36, figs. 39 and 40, and figs. 37 and 38 which represent Semon's fungus and *Glenospora khartoumensis* grown under similar conditions and on the same media.

We think that these illustrations are sufficient, without further trespassing upon Semon's kindness, to show that there is a difference between the two fungi, and this is the only point which concerns us.

We therefore submit that *G. khartoumensis*, and hence the variety of Sudanese Black Maduromycosis which we have just described, can be differentiated from Semon's Asian Black Maduromycosis (which we believe will be found to be one of Carter's Asian Black Maduromycoses), from Brumpt's African Black Maduromycosis, from Nicolle and Pinoy's African Black Maduromycosis, from Bouffard's African Black Maduromycosis, and from Pepere's Black European Maduromycosis, while the data given for Bassini's, Bovo's, Schmincke's European and Wright's American Black Maduromycoses are insufficient for purposes of definition.

We therefore believe that the Maduromycosis which we have just described is *a new form of African Black Maduromycosis*.

The following diagnostic table of the classifiable fungi found in Black Maduromycosis up to date may perhaps be useful:—

DIAGNOSTIC TABLE I OF THE CULTIVATED FUNGI IN BLACK MADUROMYCOSIS

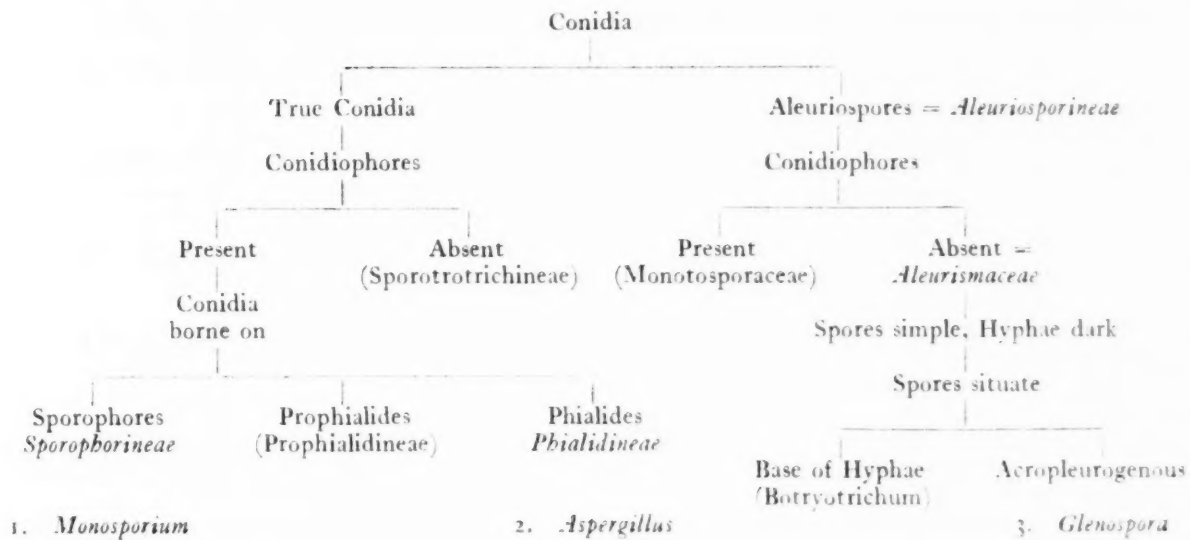
Hyphae septate, reproduction asexual by spores, sexual usually absent = *Eumycetes*

Spores not in asci or basidia = *Fungi Imperfecti*

Accessory fructifications absent = *Hyphales*

Mycelium not bacilliform (i.e. not belonging to Actinomycosis)

Spores—Conidia = *Conidiosporales*



DIAGNOSTIC TABLE II OF THE CULTIVATED FUNGI IN BLACK MADUROMYCOSIS

Hyphae septate, reproduction asexual by spores, sexual usually absent = *Eumycetes*

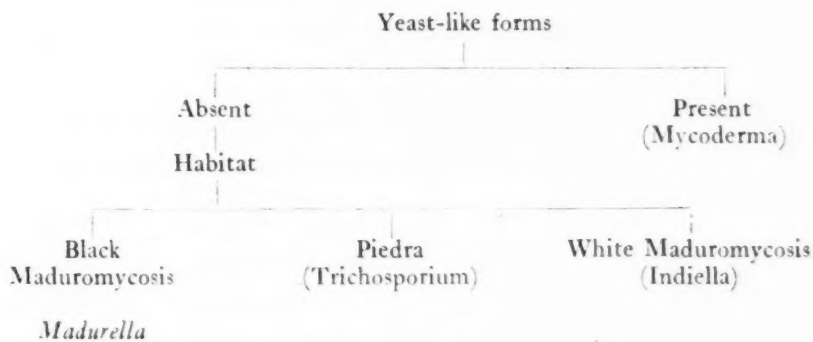
Spores not in asci or basidia = *Fungi Imperfecti*

Accessory fructifications absent = *Hyphales*

Mycelium not bacilliform (i.e. not belonging to Actinomycosis)

Spores—Thallospores = *Tballosporales*

Thallospores—Arthrospores = *Arthrosporales*



SUMMARY

We believe that we have cultivated a fungus, which we name *Glenospora khartoumensis* Chalmers and Archibald 1916 from a case of Black Maduromycosis occurring in Khartoum in the Anglo-Egyptian Sudan, and we further consider that this is nearly allied to, but not identical with, the fungus grown by Semon from a case of Black Maduromycosis coming from India.

The known causal organisms of African Black Maduromycosis are therefore:—

- I. Brumpt's Black Maduromycosis, found in Somaliland and caused by *Madurella mycetomi* (Laveran 1902).
- II. Nicolle and Pinoy's Black Maduromycosis, caused by *Madurella tozeuri* (Nicolle and Pinoy 1908)
- III. Bouffard's Black Maduromycosis, caused by *Aspergillus bouffardi* Brumpt 1905.
- IV. A Sudan Black Maduromycosis, caused by *Glenospora khartoumensis* Chalmers and Archibald 1916.

The European, American, and Asian Black Maduromycoses have already been summarized.

Diagnosis can only be effected by finding the black grains in the tumour or discharge therefrom, and then cultivating and studying the mycology of the fungus obtained from these grains. So far, the only curative treatment is excision of the growth, which when small can be easily effected, but when large may require an extensive operation or amputation of the affected member. It is important to remember that in some Black Maduromycosis the glands may become affected, and it may be necessary to remove these if enlarged.

ACKNOWLEDGMENTS

Our grateful thanks are due to Dr. Bousfield, Medical Officer of Health of Khartoum, for providing us with the specimens which we have studied; to Dr. Semon, for so generously sending us a growth from his Indian Black Maduromycosis; to Professor Saccardo, for kindly replying to our enquiries; to Professor Vuillemin, for sending us copies of his valuable publications.

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EXPLANATION OF PLATES

Most of these photographs may be examined advantageously by means of a lens, as they are all made from unbleached specimens.

PLATE IV

- Fig. 1. *Black Maduromycosis*. Longitudinal section through the length of the growth. The black grains can be seen, and also the spaces from which some, with their surrounding cells, have fallen out. $\times 1.5$ diameters. Photograph.
- Fig. 2. Section through a grain, as depicted in fig. 1, showing the relationship of the fungus to the surrounding tissues. $\times 95$ diameters. Photomicrograph.
- Fig. 3. Giant cells containing fungal tissue. $\times 140$ diameters. Photomicrograph.
- Fig. 4. Endarteritis. $\times 285$ diameters. Photomicrograph.
- Fig. 5. Degenerating cells and granules in the layer just external to the fungus. $\times 930$ diameters. Photomicrograph.
- Fig. 6. Periarteritis. $\times 650$ diameters. Photomicrograph.
- Fig. 7. Healthy cells lying just external to the fungus, which only shows as the dark mass in the upper part of the section. $\times 290$ diameters. Photomicrograph.
- Fig. 8. A polymorphonuclear leucocyte and a phagocyte, enclosing many nuclei from disintegrated polymorphonuclear cells. $\times 1,040$ diameters. Photomicrograph.
- Fig. 9. External cellular layer with supporting fibrous tissue and the innermost portion of the fibrous coat. $\times 155$ diameters. Photomicrograph.

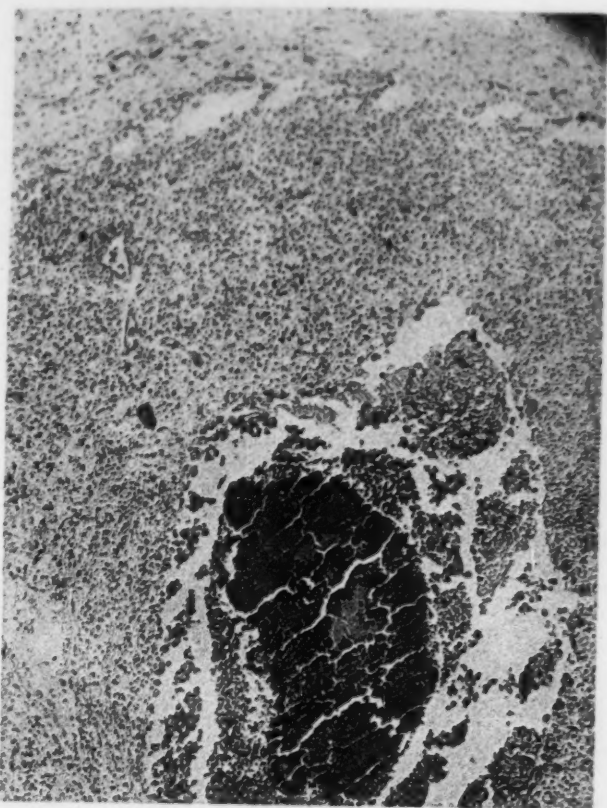


Fig. 2

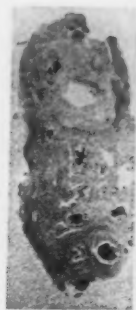


Fig. 1

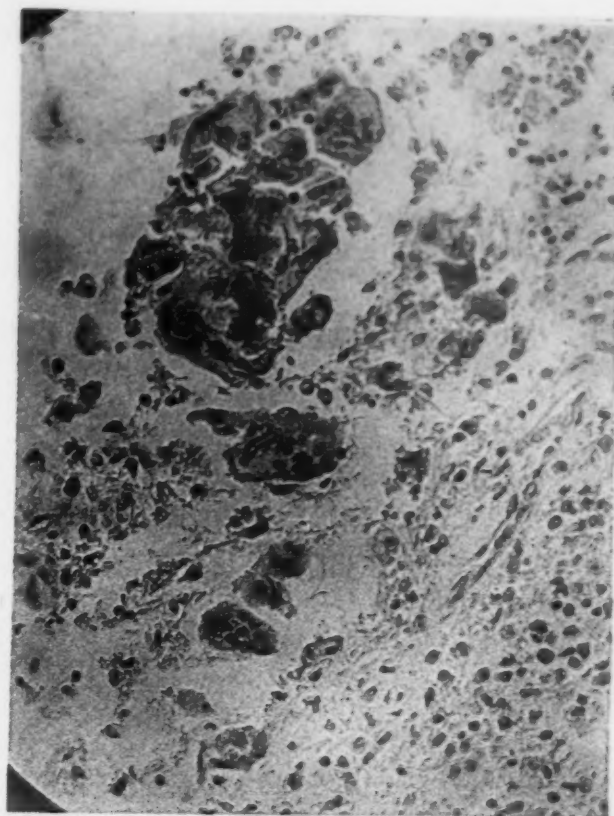


Fig. 3

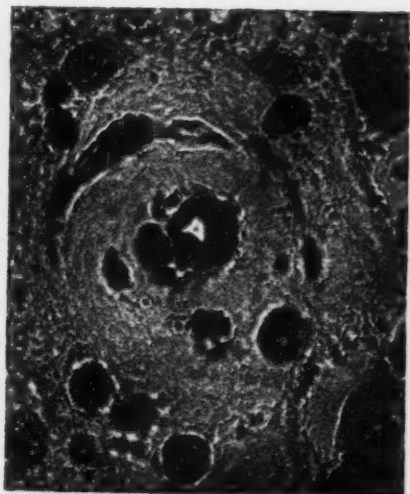


Fig. 4

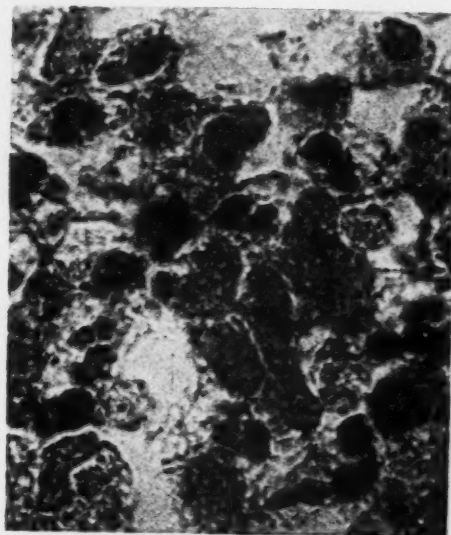


Fig. 5



Fig. 6

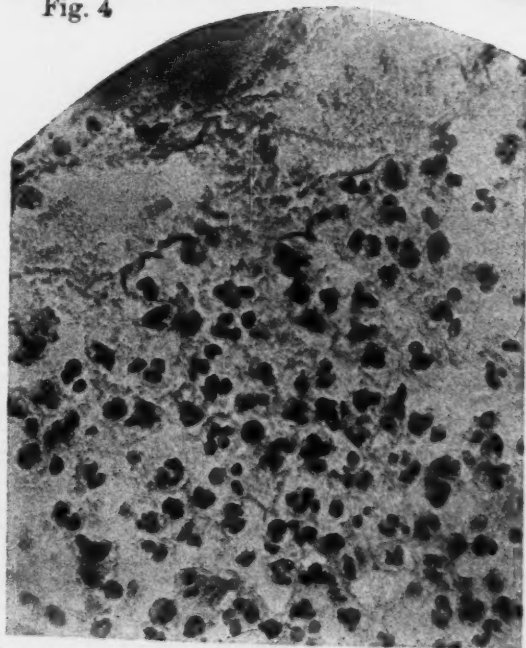


Fig. 7

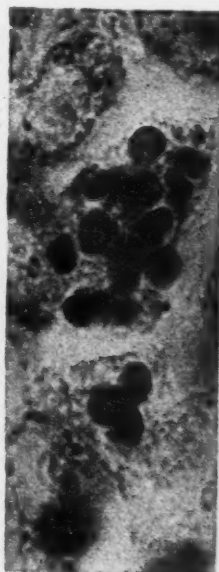


Fig. 8

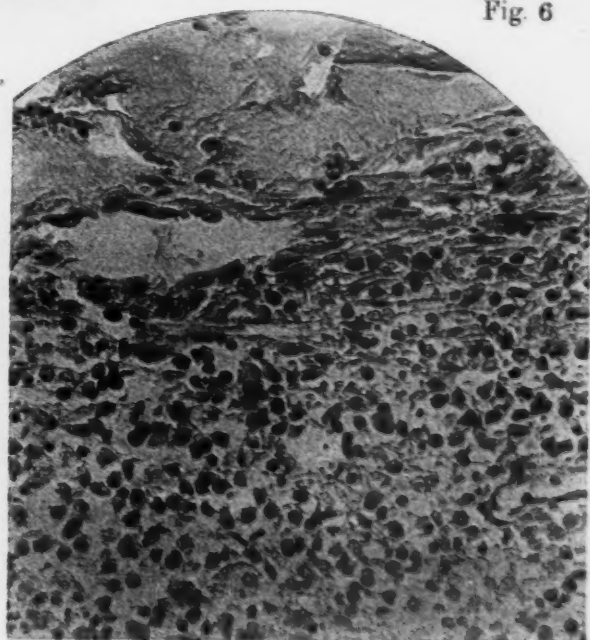


Fig. 9

A SUDANESE MADUROMYCOSIS

PLATE V

- Fig. 10. Grain showing fungal hyphae in cortical portion. $\times 250$ diameters. Photomicrograph.
- Fig. 11. Culture on Sabouraud's maltose agar (watch-glass method) for eight days at 30°C . Natural size. Photograph.
- Fig. 12. Grain showing chlamydospores. $\times 730$ diameters. Photomicrograph.
- Fig. 13. Chlamydospore containing hyaline bodies. $\times 985$ diameters. Photomicrograph.
- Fig. 14. Primary growth from a grain on Sabouraud's maltose agar for twenty-three days at 30°C . Natural size. Photograph.
- Fig. 15. Growth on Sabouraud's maltose agar at 22°C . for eleven days. Natural size. Photograph.
- Fig. 16. The same growth as that depicted in fig. 11, but magnified 4 diameters. Photograph.
- Fig. 17. Growth on Sabouraud's maltose agar at 30°C . for eleven days. Natural size. Photograph.
- Fig. 18. Growth on Sabouraud's maltose agar at 37°C . for eleven days. Natural size. Photograph.
- Fig. 19. Culture on Sabouraud's maltose agar in a Kitasato flask for eight days at 30°C . Natural size. Photograph.
- Fig. 20. Club-like hyphae as seen in fig. 10, but magnified 780 diameters. Photomicrograph.
- Fig. 21. Growth on Sabouraud's maltose agar at 40°C . for eleven days. Natural size. Photograph.

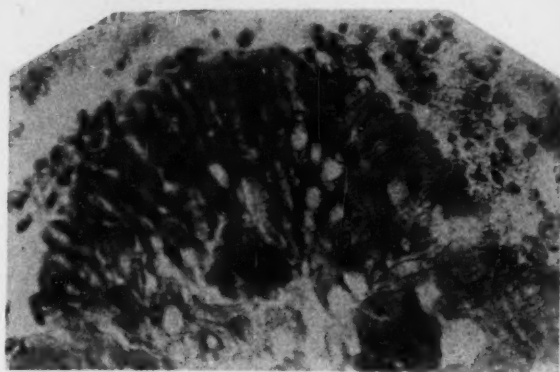


Fig. 10

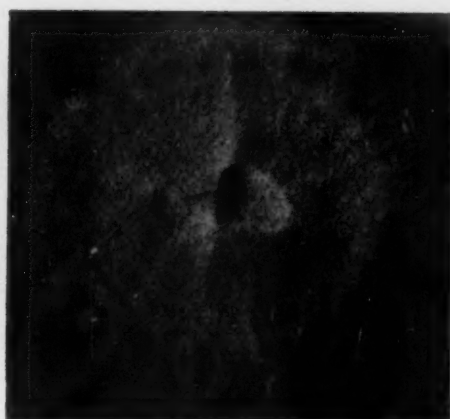


Fig. 11



Fig. 12



Fig. 13



Fig. 14



Fig. 15



Fig. 16



Fig. 17



Fig. 18



Fig. 19



Fig. 20



Fig. 21

PLATE VI

- Fig. 22. Transverse section of the grain, of which a portion is depicted in fig. 10. $\times 140$ diameters. Photomicrograph.
- Fig. 23. Central portion of an unbleached grain to show hyphae and chlamydospores. $\times 470$ diameters. Photomicrograph.
- Fig. 24. Part of the cortex depicted in fig. 10 to show a cortical hypha with bulbous peripheral end. $\times 580$ diameters. Photomicrograph.
- Fig. 25. Growth on carrot for four days at 30°C . Natural size. Photograph.
- Fig. 26. Aleuriospores, sessile on parent hyphae, and on branches, from growth in potato infusion. Living specimen. $\times 1,370$ diameters. Photomicrograph.
- Fig. 27. Aleuriospores, taken as naturally as possible to show their grouping. Note that they are sessile on the sides and at the ends of hyphae. Note that no spores are seen detached from the hyphae, even though the specimen was teased. Living specimen. $\times 400$ diameters. Photomicrograph.
- Fig. 28. Growth on potato for six days at 30°C . Natural size. Photograph.
- Fig. 29. Surface view of gelatine growth showing black masses of chlamydospores. Natural size. Photograph.
- Fig. 30. Aleuriospores at the end of hyphae, and a dark tinted segmented hypha showing the dark coloured and light coloured hyphae lying side by side. Living specimen. $\times 800$ diameters. Photomicrograph.
- Fig. 31. The branching of a fertile hypha bearing Aleuriospores. All the branches cannot be shown exactly in the same focal plane, but all details can be made out by careful examination, and the photograph is thought by us to be better than a drawing. Living specimen. $\times 750$ diameters. Photomicrograph.
- Fig. 32. Young unpigmented chlamydospores. Living specimen. $\times 730$ diameters. Photomicrograph.

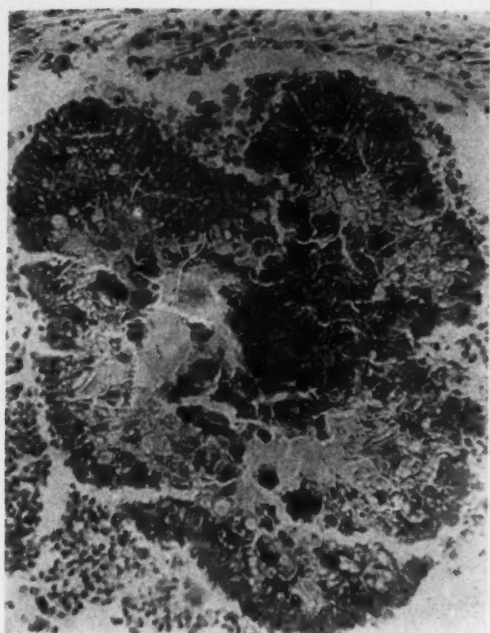


Fig. 22



Fig. 23

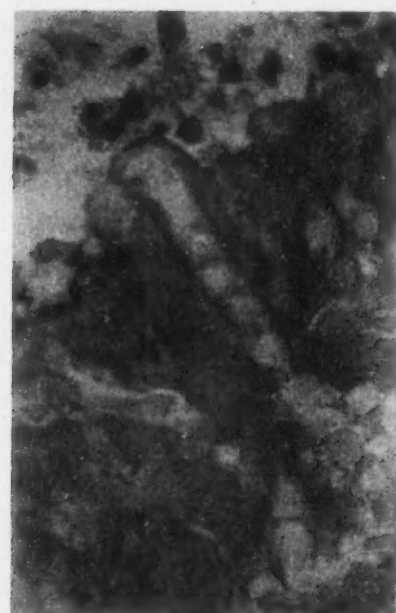


Fig. 24



Fig. 25



Fig. 26

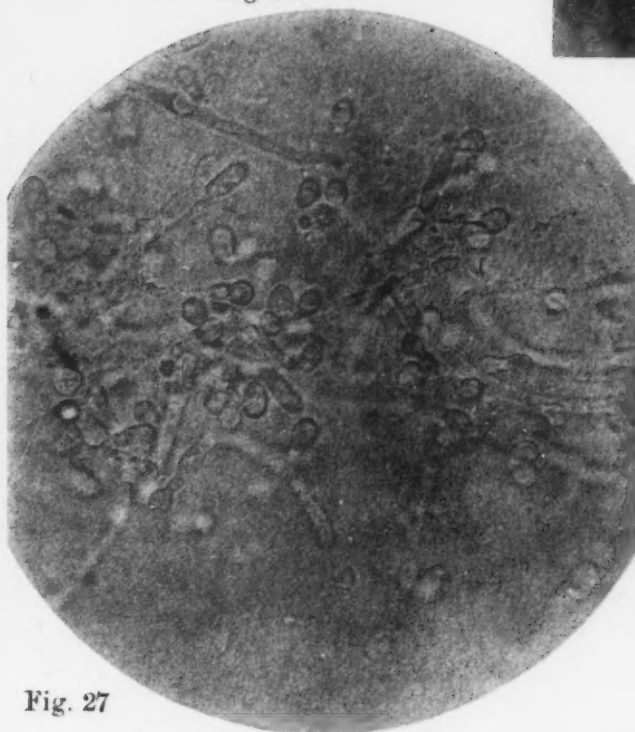


Fig. 27



Fig. 28

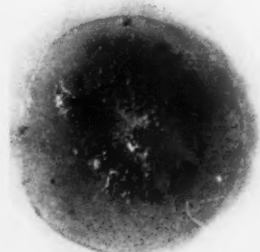


Fig. 29



Fig. 30



Fig. 31

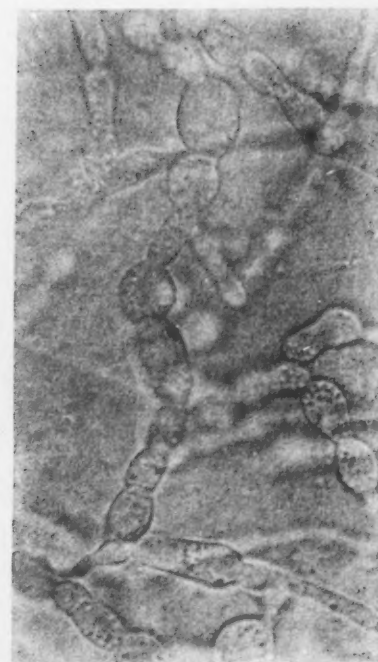


Fig. 32

A SUDANESE MADUROMYCOSIS

PLATE VII

- Fig. 33. *Semon's Indian Black Maduromycosis*. Photograph of original culture sent to Khartoum in a capped tube, and apparently on glucose agar. Natural size. Photograph.
- Fig. 34. *Khartoum Black Maduromycosis*. Grown in a capped tube for 16 days at 30° C. on maltose agar. Natural size. Photograph.
- Fig. 35. *Semon's Indian Black Maduromycosis*. Sub-culture made on clear maltose agar in Khartoum from the growth depicted in fig. 33, after twelve days' growth in an uncapped tube at 30° C. Natural size. Photograph.
- Fig. 36. *Khartoum Black Maduromycosis*. Grown under exactly similar conditions as the culture depicted in fig. 35 after twelve days' growth. Natural size. Photograph.
- Fig. 37. *Semon's Indian Black Maduromycosis*. Sub-culture on inspissated ox-blood serum after twelve days' growth in an uncapped tube at 30° C. Natural size. Photograph.
- Fig. 38. *Khartoum Black Maduromycosis*. Grown under similar conditions to fig. 37, and on the same medium. Natural size. Photograph.
- Fig. 39. *Same growth as that depicted in fig. 35*, but grown for fourteen days. Photograph.
- Fig. 40. *Same growth as that depicted in fig. 36*, but grown for fourteen days. Photograph.
- Fig. 41. *Khartoum Black Maduromycosis*. Chlamydospores from growth on gelatine. $\times 520$ diameters. Photomicrograph.
- Fig. 42. *Khartoum Black Maduromycosis*. Growth on maltose agar, showing Aleuriospores. $\times 310$ diameters. Photomicrograph.
- Fig. 43. *Khartoum Black Maduromycosis*. Growth on potato for sixteen days at 30° C. Natural size. Photograph.



Fig. 33



Fig. 34



Fig. 35



Fig. 36



Fig. 37

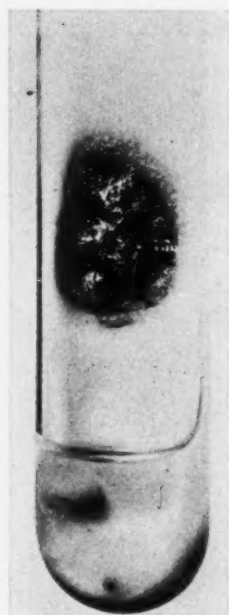


Fig. 38



Fig. 39



Fig. 40

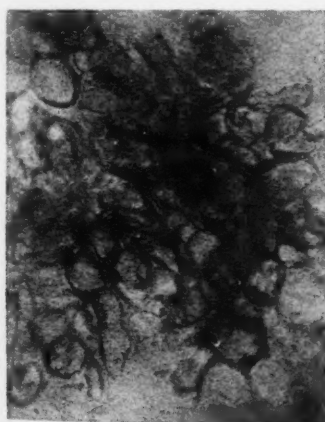


Fig. 41



Fig. 42

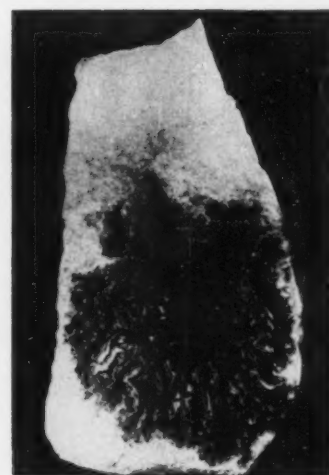


Fig. 43

A SUDANESE MADUROMYCOSIS

A SUDANESE ACTINOMYCOSIS

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PLATES VIII—XI

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INTRODUCTION

The whole subject of the organisms causing Actinomycosis, is in such a confused condition at the present time that any attempt, no matter how small, which endeavours to simplify their recognition must be of value to workers at disease in the Tropics, and therefore we bring forward the following remarks.

With Pinoy we look upon an *Actinomycosis* as forming a part of the pathological group entitled '*Mycetoma*,' of which we accept the following as a suitable definition:

'The term *Mycetoma* includes all growths and granulations producing enlargement, deformity, and destruction, in any part of the body of man, brought about by the invasion of the

affected area by certain species of fungi, belonging to different genera, which give rise to variously coloured and shaped bodies called grains, which are found either embedded in the pathological tissue forming the growths and granulations, or escaping freely in the discharge from the diseased area.'

By the term *grain* or *granum* we do not mean some vague body which may be confused with the mycological expression '*Sclerotium*'; on the contrary, we use these words in the sense defined by Chalmers and Archibald, which is as follows:—

'The word *granum* or *grain* is applied to certain coloured bodies of varying consistence, size, and shape, which are composed of hyphae, and sometimes chlamydospores, embedded in a matrix, and which on germination give rise to mycelial filaments and are found in Mycetomas.'

By the above definitions it is possible to differentiate between the *Mycetomas* and those *Pseudomycetomatous* conditions which are not infrequently seen in the Tropics, and are due to *Framboesia tropica*, *Sporotrichosis*, and *Angiokeratoma*.

The above definition of a Mycetoma covers very wide pathological and aetiological fields, and for purposes of simplification it is suitable to use Pinoy's classification, and to divide the Mycetomas into two classes, as follows:—

- A. *The Maduromycoses* are those forms of *Mycetoma* with grains composed of large segmented mycelial filaments, possessing well-defined walls, and usually chlamydospores.
- B. *The Actinomycoses* are those forms of *Mycetoma* with grains composed of very fine non-segmented mycelial filaments, in which usually the walls are not clearly defined from the contents, and without chlamydospores.

The term *Pseudoactinomycosis* is used very loosely, but should mean an infection of man due to a *Nocardia* or *Cohnistreibothrix*, which does not come under the definition of an actinomycosis as given above.

In the present communication we are only concerned with the Actinomycoses, of which we propose to discuss a variety found in the Sudan, but before so doing we desire to consider, in the briefest possible manner, the Actinomycoses previously described in man.

HISTORICAL

For purposes of description the history of the Actinomycoses of man may conveniently be divided into four periods, which are :

- A. Early History.
- B. Madura Foot Period.
- C. Mycetoma Period.
- D. Actinomycosis Period.

A. *Early History.* According to Waring as quoted by Collas, the Sanscrit work 'Vaweda,' by which is probably meant At'harvavéda, describes a disease of the foot termed *Padavalmicum* which causes swelling and the formation of little fleshy tumours which, after an interval of a year from the commencement of the disease, discharge a peculiar fluid.

This disease is distinguished from another malady of the foot which is called *Slipatham*, or Elephant Foot.

If the above is a correct quotation from the At'harvavéda, then the Ancient Indian Surgeons must have distinguished Elephantiasis of the foot from such conditions as might have been produced therein by Mycetoma, Yaws, etc.

It is, however, curious that, like Collas, we have been unable to find any account of such a disease in the writing of Suśruta.

The term *Perical* used by Kaempfer in 1712 is applicable to any enlargement of the foot, whether caused by Elephantiasis, Mycetoma, or Yaws, but the Pondichèry Missionary of 1714 appears to have seen the disease *Mycetoma*, and possibly the *Actinomycotic* variety, because he describes under the term 'Fourmilière des Vers' an incurable disease of the foot in which numerous small ulcers form which intercommunicate by means of canals full of worms. These canals are described as being peculiar in that if one closes another opens.

Heyne probably recognised some sort of a Mycetoma, in 1806, in the foot of the Rajah's brother at Cuddapah, and Brett's 'Adipose Sarcoma,' described in 1840, may have been of the same nature, but we have been unable to refer to these descriptions with which the early history of the diseases closes.

B. *Madura Foot Period.* With the closing years of the last period it will be noticed that it began to dawn upon the medical

men of India that there existed in that country a peculiar disease of the foot, and this was emphasized by Gill of Madura, who, in 1842, described a condition of that member which was characterised by marked deformity and fungoid excrescences, from which flowed an offensive ichorous discharge, while internally the disease produced a condition resembling fibro-cartilage, and destroyed joints, cartilages and ligaments.

Four years later, Colebrook, Gill's successor at the Madura Dispensary, confirmed these observations, and stated that the disease was commonly known in some parts of India as 'Madura Foot.' As no mention is made, as far as we know, by these authors of any black pigment being present in their cases, we conclude that probably they saw the actinomycotic variety of Mycetoma.

It is curious to note that about this time (1845) von Langenbeck, in Kiel, made illustrations of some curious bodies which he considered to be fungal in nature, and which he found in the pus from a case of Spinal Caries. Unfortunately he never published this observation, which was made known by Israel one year after Bollinger's discovery, which will be mentioned below.

In 1848, Lebert found some peculiar spherical yellowish bodies, about the size of a pin's head, in some thick gelatinous pus which Louis had obtained from an abscess associated with much swelling of the thoracic wall in a man aged 50 years, in Paris. These bodies were carefully examined, both microscopically and chemically, and drawings were made which were subsequently published by Lebert (1857).

We have examined copies of these drawings, and they represent in a typical manner the fungus of an Actinomycosis. Lebert, however, failed to recognise their fungal nature.

In 1855, Smith, in London, made some drawings for Paget of a tumour of the upper jaw, in which an organism resembling a ray-fungus is portrayed. These drawings were published by Kanthack (1896).

Also in 1855, Ballingall, in India, described a disease of the foot in the discharge from which he found bodies composed of large cells with transparent fringes containing irregular spicules, or simply of radiating spicules without cells. In 1858, Rustomji described a variety of Madura foot in which he found small, soft, yellowish granules, and which he distinguished from another variety

of the same disease in which he found a dark, soft, thick substance.

The description of Black Maduromycosis by Godfrey in 1845, and of Actinomycosis by Ballingall in 1855, combined with the differentiation of these two forms of *Madura Foot* by Rustomji in 1858, closes the historical era which we have named the Madura Foot Period.

C. *Mycetoma Period*. In 1860, Vandyke Carter began that series of epoch-making publications which were eventually to lay the foundations of the fungal nature of the black and yellow varieties of Madura Foot. He first became acquainted with the black, subsequently with the yellow, and finally with the pink or red varieties of the disease.

He recognised the fungal nature of the black grains, and inventing the term Mycetoma from the Greek words *μύκης* a fungus and *οἶδημα* a tumour, he named the two principal varieties *Melanoid* and *Ochroid* respectively, and he believed them to be different stages of one disease caused by a mould *Chionyphe carteri* Berkeley 1862, belonging to the Mucorini, which he obtained by placing black grains on cotton soil or rice, although he honestly stated that the fungus had no clear connection with the black grains, but seemed to spring up independently of them. Eventually in 1886, influenced no doubt by the researches which will be detailed below, he gave up *Chionyphe carteri*, and drew attention to the similarity existing between the by-then-known fungus of Actinomycosis and that found in Mycetoma. It may also be noted in passing that Coquerel (1866) in Réunion described and illustrated a case of the Ochroid variety in a man coming from Pondichéry; that Rivolta found rods like those in the retina in 'Mal del Rospo' in cattle in Italy; that Moxon and Hogg, in 1870, noticed fine fibrils in the Ochroid grains of a Mycetoma; that Robin, in 1871, saw crystalline concretions in some pus which he compared with Lebert's illustrations; that Heller, in 1872, observed and described some ray-like bodies in yellow grains from an acute infection in man, and that Perroncito, in 1875, described granular elements considered to be cryptogamic in nature in yellow grains of the Osteosarcoma of the jaw in cattle. All these observations form a natural series leading up to the discovery of the true nature of the causal organism, and thus bring us to the last period of our history.

D. *The Actinomycosis Period*. This period opens with

Bollinger's epoch-making work in 1876 on the lumpy-jaw of cattle, a disease which had been recognised since 1785, and in which he found the constant presence of a branching organism. This fungus was examined by Harz (1877-78), who gave it the name *Actinomyces bovis*, but, most unfortunately, this generic name cannot stand, because, unbeknown to Harz, it had already been used by Meyen (1827) for a fungus which he called *Actinomyces horkelii*, and which is in no way related to the group of fungi which we are considering. This mistake launched the generic name applicable to these organisms on to a sea of change, and led to much confusion.

Genus Nocardia. Bollinger's ray-fungus belongs to a genus of which the correct name is *Nocardia* de Toni and Trevisan 1889, a term derived from Nocard, the celebrated French Parasitologist, who was the first investigator to clearly recognise this fungus in France. We state that it is the correct name for the following reasons:—

1. It is the oldest name against which no objections can be raised.
2. It has been formally adopted by the Botanical Section of the First International Congress of Pathology.
3. The objections to the other names in use are as follows:—
 - (a) *Streptothrix*, as proposed by Rossi-Doria, cannot be used, as it was originally suggested by Corda in 1839 for *S. fusca*, which is quite a different fungus.

It was also used in 1875 by Cohn for another organism closely allied to a 'Nocardia,' as we shall see later, so that Cohn's and Rossi-Doria's names can only be utilised as synonyms of the organisms to which they were wrongly applied because of the priority of Corda's name.

- (b) *Discomyces* was used by Rivolta in 1878 merely as a trivial name, and though it has not been applied to any other genus, still the word *Discomycetaceae* was introduced in 1836 by Fries for a large fungal family and has come into general use, and therefore has the double claim of priority and general use, and as its type genus should bear the name *Discomyces*, confusion is

bound to arise if the same term is retained as the generic name of Bollinger's organism.

- (c) *Bacterium* was suggested by Affanasieff in 1888, but Ehrenberg had used this name in 1830 for the organisms popularly known as bacteria, and therefore Affanasieff's suggestion falls to the ground.
- (d) *Oospora*, as utilised by Sauvageau and Radais in 1892, is not available because it is younger than the name 'Nocardia,' and because it was previously used in 1833 by Wallroth for certain fungi previously classified as *Torula* Persoon 1801.
- (e) *Cladothrix*, as brought forward by Macé in 1897, cannot be used because the name 'Nocardia' has priority, and because it was originally used by Cohn in 1875 for the organism *Cladothrix dichotoma*, which is septate and is only falsely branched, and hence is quite different from Bollinger's fungus.

The genus *Nocardia*, which will be defined later in this paper, contains a large number of species which live saprophytically in soils from whence their spores can be spread by the agency of air or water to sewage, sputum, etc., etc. Some of them have acquired parasitic habits, living in plants in which they cause root tubercles or, in other instances, tumours with ray fungi, thus somewhat resembling the Actinomycosis of animals. They have also been found living in Molluscs and in the alimentary canals of larval insects, as well as in the form of pathogenic fungi in Reptilia, Aves and Mammalia, in which they mostly occur in the Herbivora or in Omnivorous Man, though they are known in the grass-eating dog, but are rare in other Carnivora. Their geographical distribution appears to be world wide.

With reference to their method of entry into the human body, it appears to be often associated with some slight traumatism with some vegetal substance, such as a thorn, while the best treatment is undoubtedly complete removal wherever possible, still partial extirpation associated with treatment by Iodide of Potash, as first advocated for this purpose in 1885 by Tomassen, but in large doses such as ninety grains per diem, as used by Carroll with success in 1905, is also capable of effecting a cure.

For purposes of comparison with the fungus which we are about to describe as the causal agent of a new form of Actinomycosis, it is necessary to briefly review the organisms at present known to cause this disease in man, and we will commence with Bollinger's cattle parasite.

1. *N. bovis*. The correct name for Bollinger's organism is *Nocardia bovis* (Harz 1877) and it appears to have been first seen in man by Israel in 1878. Corre (1883) was the first to draw attention to the similarity between Actinomycosis and the Ochroid variety of Mycetoma, while Acland (1886) was the second observer to demonstrate the presence of Actinomycosis in man, and as Israel's name is associated with quite a different human Actinomycoses, we propose to name this variety *Acland's Actinomycosis*. In 1886, Vandyke Carter, as we have already stated, also drew attention to the likeness between Actinomycosis and Mycetoma. Finally, in 1891, Bostroem grew *N. bovis* from eleven cases of Actinomycosis in man, and since that time it has often been cultivated and described. It is a *Nocardia* with radially arranged filaments which show club-like enlargements of their extremities, caused by a protective thickening of the walls in animals and less commonly in man, and having abundant Gram-positive but not acid-fast hyphae, some of which end in chains of arthrospores.

It grows well aërobically at 22°C., but better at 37°C. Anaërobic growths are, as a rule, but poorly developed.

It may form a dry pellicle on the surface of broth, but more usually it gives rise to cohering colonies at the bottom of the tube, and in either case the medium remains clear.

It grows slowly on gelatine, producing a yellowish-white growth and slow liquefaction, beginning about the seventh day. The resulting fluid may or may not be dark coloured. On blood serum it produces poor growths, and no liquefaction or pigmentation of the medium.

On agar and glycerine agar it forms hard spherical white colonies, which give rise to an undulating crateriform growth, having a yellowish or greyish tint, which in its turn becomes a lichenoid ashen grey or yellowish mass with a powdery efflorescence. On maltose agar it forms discrete fawn-coloured colonies, later becoming yellow, dark brown or even black, while the medium may be slightly darkened.

On potato it forms confluent, hard, raised, variously-coloured masses, at first white but becoming greenish yellow, brown, greyish black or even black, with more or less erosion and pigmentation of the medium to which the growth is very adherent. No diastatic action has been observed.

Litmus milk is first reddened, but later it becomes a clear brown alkaline liquid. It is pathogenic for man, ox, horse, pig and other animals, while experimentally rabbits and guinea-pigs have been infected by intra-peritoneal inoculation.

2. *N. asteroides*. *N. bovis* (Harz 1877) is not the only organism known to cause Actinomycosis in man, for in 1890 Eppinger obtained an organism which he called *Cladothrix asteroides*, and which is now known as *Nocardia asteroides* (Eppinger 1890), from the lesions in a case of pseudo-tuberculosis of the lungs and pleura, with old caseous nodules in the apices and calcareous degeneration of the bronchial and supra-clavicular glands, together with a cerebral abscess which had ruptured into the ventricles. The fungus was Gram-positive and acid, but not alcohol fast, and grew aërobically on laboratory media, and was pathogenic for laboratory animals. It was afterwards recognised by Almquist, in 1890; by Sabrazès and Rivièrè, in 1894; by Aoyama and Miyamoto, in 1900, in Tokio; by MacCallum, in 1902, in America; and by Schabad, in 1903, in Russia. It is also the same as the fungus described by Musgrave and Clegg (1907) in a case of Mycetoma in the Philippine Islands under the name *Streptothrix freeri*, because in 1908 these authors state:—

‘It is identical with Eppinger’s organism—and the name given by us in the first publication should fall as a synonym for *S. eppingeri*, the latter having the priority.’

In order to understand this quotation, it is necessary to remember that *S. eppingeri* is a synonym of *N. asteroides*.

In 1909, Lindenberg, in Brazil, isolated a fungus from a case of Mycetoma of the left leg which began in the popliteal space, and to this organism he gave the name *Discomyces brasiliensis*.

He was very careful to separate it from *N. bovis* and from *N. madurae* (*N. indica*), but he does not appear to have done so with regard to *N. asteroides*. We therefore offer a comparison between the two organisms in the following table:—

Nature of test	<i>N. asteroides</i> from Musgrave and Clegg	<i>N. brasiliensis</i> Lindenberg	Result of comparison
Seat of disease	Mycetoma of foot	Mycetoma of leg	Difference unimportant
Grains	Consistency : dough-like Colour : yellowish white Size : 0.25-0.5 mm. in diameter	Consistency : soft Colour : yellowish white Size : 0.1-0.5 mm. in diameter	No important difference
Clubs	Usually absent	Absent	Agree
Bacillary and coccal forms	Numerous bacillary and coccus-like varieties	Bacillary and coccal forms present	Agree
Optimum temperature ...	Slower growth at 30° C. than at 37° C.	Better growth at room temperature than at 37° C.	Slight disagreement
Anaërobic cultivation	Does not grow	Does not grow	Agree
Broth	Floating flat particles which later fall to the bottom Medium not affected	Small particles which later fall to the bottom of the tube Medium not affected	Agree
Gelatine	No liquefaction	No liquefaction	Agree
Sabouraud's glucose agar at 37° C.	Centre yellow, periphery pink to pinkish white	Colonies rose violet	Slight disagreement
Potato	At first delicate pink, and later yellow ochre centre with pinkish or white periphery ; the medium becomes darkened	At first a rose colour, and later a yellow orange colour ; the medium becomes brown	Agree
Serum	Growth slower Colonies at first white, later diffuse pink	Grows very badly at 37° C. Colonies white	Later pink not mentioned in <i>N. brasiliensis</i>
Milk.....	Yellowish mass No coagulation	Yellowish orange pellicle No coagulation	Agree

The inoculations into animals are not comparable, as Lindenberg did not use monkeys. He was unsuccessful with a guinea-pig, but does not say how he inoculated it, while Musgrave and Clegg were successful by means of intraperitoneal inoculations.

The differences as set forth above between *N. brasiliensis* and *N. asteroides* appear to us to be very slight, and therefore we are able to agree with Pinoy in his belief that they are one and the same organism.

Also Cranwell, Bachmann, and Del Pont (1909) gave an excellent and well-illustrated description of a yellow Mycetoma in Buenos Aires. Unfortunately, they did not grow it on inspissated blood serum, but, as far as we understand their account, we should classify this organism, which they did not name, as *Nocardia asteroides*.

Nocardia asteroides possesses Gram-positive, acid but not alcohol-fast hyphae, which are without club-like enlargements. It produces restricted growths aërobically and usually anaërobically at 22° C. and 37° C., but nothing is stated in the literature we have consulted with regard to any odour arising from these cultures. It does not liquefy gelatine or blood serum, nor has it any diastatic action. It reddens litmus milk, which later becomes alkaline, but is not coagulated or cleared. It grows on the agars and on potato, producing reddish (often brick red) growths. It is pathogenic for monkeys, rabbits, and guinea-pigs.

3. *N. liquefaciens*. This fungus was obtained by Hesse in 1892 from a man in Germany with a left inguinal abscess which communicated with the rectum. Subsequently other abscesses formed on either side of the dorsal spine. The pus from these abscesses discharged soft yellowish grains about the size of a millet seed, which contained a Gram-positive fungus which did not possess clubs. On cultivation it grew readily, and was found to be strictly aërobic. In gelatine stabs it formed a nail-shaped growth, which at room temperature in Europe was only visible on the third day, while liquefaction, beginning on the fourth or fifth day, was complete by the end of the week. The liquefied gelatine was not discoloured, and if the growth stuck to the glass it was yellowish with a whitish covering. On blood serum it formed small cloudy granules of the same colour as the medium, in twenty-four to forty-eight hours. Liquefaction begins at the end of the first week and proceeds slowly,

the liquid remaining quite clear and colourless, and only after some six months turning to a reddish-yellow colour. In broth it forms delicate flakes which fall to the bottom of the tube, and consist of a lower surface which is yellowish white, and an upper surface which is snow white. The medium remains quite clear. No surface growth is mentioned.

On agar the colonies at first form separate rosettes, which remain distinct for a time. These colonies appear to resemble the gelatine culture, being yellowish below and having a white envelope. The growth on glycerine agar is more vigorous than on ordinary agar.

On potato it forms small yellow nodules by the second day, which later become covered with a snow-white efflorescence, which does not alter. Apparently it was not grown on glucose agar, milk or eggs. Intravenous, intraperitoneal and subcutaneous injections into rabbits, guinea-pigs and white mice were negative.

Hesse gave it the name *Cladothrix liquefaciens*, which now becomes *Nocardia liquefaciens* (Hesse 1892), and it appears to be the same organism as that named *Streptothrix buccalis* by Goadby in 1903, and found by him in 1899 in the mouth in cases of pyorrhoea. Goadby's form showed clubs, or club-like swellings. It precipitated the casein in milk, which became clear.

4. *N. indica* (Kanthack 1893), studying specimens of Black and Yellow Mycetoma which came from India, concluded that the latter variety was a true Actinomycosis, and attempted to show that the former was the same, only in a degenerated condition.

He only examined the specimens microscopically, as no cultures were possible, and named the fungus in question *Oospora indica* Kanthack 1893, calling his two varieties *O. indica* var. *flava* and *O. indica* var. *nigra*. The name of this fungus, translated into more modern nomenclature, becomes *Nocardia indica* (Kanthack 1893).

Boyce and Surveyor (1894) clearly proved that the Melanoid variety was due to quite a different fungus from that causing the Ochroid variety, which latter they considered in some, but possibly not in all, cases, to be an Actinomyces, a conclusion in which they were supported by Hewlett and by Boccaro. The latter analysed one hundred cases of Madura Foot, of which the vast majority were Black Mycetomas, while seventeen had evidence of pricks with an

acacia (Babul) thorn, in several of which it was found present on examination.

Kanthack's name appears to have been overlooked, but it certainly has priority as regards the fungus of an actinomycotic nature causing the Ochroid variety of Mycetoma as seen in India, but difficult of recognition in that it was not cultivated.

In 1892, Gémy and Vincent described a parasitic disease of the foot in Algeria, which they considered analogous to, if not identical with, the Ochroid variety of Vandyke Carter's Mycetoma.

In 1894, Vincent, still working in Algeria, met with a *Streptothrix* in a similar case. This fungus, which was first known as *Streptothrix madurae* Vincent 1894, is believed to be identical with the fungus found by Boyce in London in an agar tube inoculated in India from a case of the Ochroid variety of Mycetoma.

This *Streptothrix* found by Boyce is of course an entirely different organism from the mucor-like fungus called *Chionyphe carteri* mentioned above, which therefore cannot be placed in the list of synonyms of *N. madurae*, as has been done by some authors.

Boyce's culture showed a fungus without club-shaped extremities which grew very slowly on agar, glucose-agar and glycerine-agar, at a temperature varying between 35° C. and 37° C. No formation of pigment was observed, but it was remarked that the organism closely resembled that of Actinomycosis.

In 1904, Cornwall reported the cultivation of Vincent's organism in India. He washed the grains in six changes of sterile salt solution, and then planted them on agar in tubes. After an interval of one or two months a growth appeared, which in some cases assumed a pink colour and in others remained a dull white. In subcultures it grew more freely, preserving its characteristics, one of which was to adhere so closely to the medium that each nodule had to be literally dug out when it was required to transfer it to another tube. Puff balls formed in broth and hay infusions, while it was noted that the fungus required plenty of oxygen for its growth and only occasionally formed the pink pigment.

This description by Cornwall leaves no doubt in our minds that he met with Vincent's organism in a case of the Ochroid variety of Mycetoma, and if this is correct, then Kanthack's name assumes its priority and Vincent's becomes a synonym, and the correct name of

the fungus is *Nocardia indica* (Kanthack 1893), and this is supported by Strong's culture of the same fungus from an Indian Mycetoma in 1908.

With regard to the remaining history of the fungus, it should be noted that in 1898 Legrain, and in 1899 Brault, again described its presence in Algeria, while in 1901 Albertini and Desvernine reported its presence in Cuba, and in 1902 Brumpt discovered that it existed in Abyssinia, while Sommer y Greco demonstrated its presence in the Argentine in 1904, and Williamson in Cyprus in 1905, in which year Brumpt, in his classic on Mycetomas, stated that he had obtained it from India, Somaliland and Senegal.

In the same year, Pelletier described a case of Mycetoma with red grains which he saw in Saint Louis, in Senegal. The grains were very small, from 0.4 to 0.5 of a millimetre in diameter, and of a beautiful vermilion red colour. In the same year, Laveran published a paper upon Pelletier's Mycetoma, in which he says that it was possible on making sections of the tumour to easily discern therein little red spots of variable size which stood out from the surrounding neoplasm. These grains contained a large number of Gram-positive micrococcal-like bodies embedded in a ground substance. These bodies, which measured 0.7 microns in diameter, were never found isolated, but always in masses or short chains. No trace of a mycelium could be seen, and for this reason he gave it the name of *Micrococcus pelletieri* Laveran 1906. But coccal-like forms are commonly found in Nocardial infections, and in 1912 Thiroux and Pelletier reported that this red Mycetoma was fairly common in Senegal, where one of them had met with eight cases, from one of which, a suppurating tumour of the right side of the chest, they obtained cultures on Sabouraud's gelatine which very much resembled those of *N. madurae*, but differed therefrom in the following particulars:—

1. The growths were ruby red from their commencement.
2. It had only so far been grown on Sabouraud's gelatine.
3. The growths did not penetrate into the gelatine, and were easily detached.
4. In the parasitic stage the organism takes the form of a *Micrococcus* in Zooglea.

They renamed the parasite *Oospora pelletieri*. In the discussion

on this paper, Laveran agreed with Thiroux and Pelletier's finding, and Pinoy pointed out that the only real differences between it and *N. madurae* were the greater intensity of the red colour and the more abundant sporulation. Further, he suggested that the correct name was *Nocardia pelletieri*. Under these circumstances, *N. pelletieri* becomes simply a synonym of *N. indica*, of which the full list of synonyms will be given later.

Clegg and Hobdy (1916) described *N. indica* in a native woman in Hawaii.

Nocardia indica, with yellow or red grains, possesses Gram-positive but not acid-fast hyphae, without clubs. It forms restricted growths under aerobic surroundings at 22° C. and 37° C., but will not grow under strict anaerobic conditions. The cultures are without any distinct odour. It is usually said not to liquefy gelatine or blood serum, but Koch and Stutzer say that it has a peptonising effect after a long time. Milk is not coagulated, but after some time is cleared. Pinkish colonies are produced on the agars and on potato. It is non-pathogenic for animals, as far as is known.

5. *N. garteni*. Garten (1895) met with an organism in cases of Actinomycosis in man which he called *Cladothrix liquefaciens* No. 2, in order to distinguish it from Hesse's fungus, which he called *Cladothrix liquefaciens* No. 1, but Brumpt, in 1910, altered Garten's name to *Discomyces garteni*, which now becomes *Nocardia garteni* (Brumpt 1910).

This fungus was grown in 1895 by Garten from the lesions of a case of necrosis of vertebrae and ribs, which was associated with abscesses, sinus formation and empyema. The grains were composed of a tangle of ramified filaments without club formation.

The organism was an aerobe which grew easily on various media, producing on gelatine fine greyish-white points. On the fourth day liquefaction commenced, and was completed by the eighth day. Nothing is said as to the liquid being coloured in any way, and, therefore, we must assume that it was not tinted. On agar, glycerine and glucose agar it formed a greyish-white growth, which became somewhat wrinkled on the surface after two to three days. The wrinkles are deep folds on glycerine agar.

On serum it forms a white layer, which becomes wrinkled and folded after forty-eight hours, when commencing liquefaction may

be noted. On the third day the liquid has increased considerably, and by the sixth day the whole serum is reduced to a perfectly clear fluid. On potato it gives rise to white colonies, while the surrounding medium becomes greenish in colour. It apparently was not grown on eggs, milk, broth, or peptone solutions. It is pathogenic for rabbits and guinea-pigs.

6. *N. krausei*. In 1899, Krause found an organism in an abscess of the lower jaw, in a man in Germany, which was characterised by having long and short rods and club-like forms resembling the diphtheria bacillus.

It did not grow at 22° C. nor on gelatine or potato, but it was a facultative anaërobe which formed slightly yellowish colonies on glycerine agar and was not pathogenic for rabbits, guinea-pigs or mice.

This fungus was named *Streptothrix krausei* by Chester 1901, which name has become changed to *Nocardia krausei* (Chester 1901).

Allied to, or identical with, this species are the fungi causing the conditions described by Mosetig-Moorhof, Dor, and Poncet, and often called 'Pseudo-Actinomycosis' or the Mycoses with yellow grains, which are larger than those of the ordinary Actinomycosis, while they are less numerous in the pus. Microscopically they show a tangle of filaments longer and larger than those of ordinary Nocardias, between which lie micrococcal-like débris. They never show clubs at the periphery and do not grow on solid media like gelatine. They grow quickly in broth, forming a skin on the surface. Cultures on serum give clavate forms like the diphtheria bacilli.

The fungus causing the above conditions was named *Nocardia ponceti* by Verdun in 1913, and may be a synonym for *N. krausei* (Chester 1901) for the following reasons:—

A. The Pseudomycetomatous condition of Poncet does not differ from the definition of Actinomycosis given at the commencement of this paper.

B. *N. ponceti* only differs from *N. krausei* in the following details.

1. *Broth is rendered turbid and has a bad odour*, but Foulerton has pointed out that this turbidity, together with the odour which was described as being associated

with these growths, may have been due to the pus not being collected aseptically, and therefore the turbidity and odour may have been due to contamination, as in addition to these characters *N. ponceti* forms a typical puff ball, just like *N. krausei*, in which the turbidity and odour are absent.

2. According to Verdun, it does not grow on agar. It is not known whether *N. krausei* grows on plain agar, but it can grow on glycerine agar and (according to some authors) on glucose agar.

C. They resemble each other in:—

1. Morphology.
2. They both possess clavate forms like the diphtheria bacilli.
3. Both grow on serum.
4. Neither grows on gelatine.

Other reactions are given for one, but not for both organisms, and are therefore useless for purposes of comparison.

We therefore at present see no reason why *N. ponceti* should be considered as a species distinct from the older *N. krausei*, of which its name becomes a synonym.

7. *N. somaliensis*. Bouffard observed two cases of a Mycetoma at Djibouti in French Somaliland, which appears to be peculiar both in its histological appearances and in its cultural characters. Brumpt, in 1906, classified this fungus in his new genus, *Indiella* Brumpt 1906, calling it *I. somaliensis*, and pointing out that, judging by the descriptions given by older writers in India of the macroscopical appearances of some of the Ochroid varieties of Mycetoma, this variety might be found to be more common than Vincent's *N. madurae* (= *N. indica* of Kanthack).

Balfour (1911) reported the presence of the same causal agent in a case of Mycetoma of the hand in the Anglo-Egyptian Sudan, and gave a photo-micrographic illustration of the growth, and in the same year Fulleborn described and gave excellent illustrations of a case from German South-West Africa, which occurred in a Herero aged twenty years. A study of Fulleborn's preparation induced Brumpt to alter his generic diagnosis for the fungus which in 1913 he classified as *Discomyces somaliensis*, which, converted into our

present nomenclature, becomes *Nocardia somaliensis* (Brumpt 1906), but he is inclined to think that it ought to form a separate genus or sub-genus, for which he proposes the name *Indiellopsis* Brumpt 1913, because it secretes around itself in the grain a hard sheath, insoluble in potash and in Eau de Javelle, which no other *Nocardia* is known to do.

This year, we met with this fungus in a *Mycetoma* of the foot, in Khartoum.

The grains are hard, one millimetre in diameter, and being of a reddish yellow colour resemble the eggs of fish. The fungus will not grow on hay, or on dura broth, but it quickly produces a white lichen-like folded growth, becoming yellow on the fifth to sixth day on potato, but this growth never becomes red like that of *N. indica*.

Genus Cohnistreptothrix. In 1891, Wolff and Israel published a beautifully illustrated account of a *Streptothrix*, which they had isolated from two cases of Actinomycosis in man, viz., from the lungs and from a retromaxillary growth. This organism was considered to differ from *N. bovis* in that it grew best anaërobically, that branching was absent, and that its injections into animals were regularly positive in their result. These three characteristics induced Kruse, in 1896, to make a new species for it under the name *Streptothrix israeli*. In 1911, for reasons presently to be set forth, Pinoy founded a new genus *Cohnistreptothrix*, with Israel's organism as the type species, and therefore its name becomes *Cohnistreptothrix israeli* (Kruse 1896).

It appears to us to be of importance that the reader should clearly understand the nature of the organisms included in this genus, and, therefore, we digress from our main subject in order to give a brief history.

Lachrymal concretions have been known since Césolin described them in 1670. In 1848, Gruby, examining one of these objects, found it to be composed of a fungus, which he believed to be the same as that causing favus, but Cohn, in 1875, examining another such concretion, also saw a fungus, for which he created a new genus *streptothrix*, calling the fungus in question *Streptothrix foersteri* Cohn 1875, which may be the same organism as *S. aureus* Du Bois de Saint Séverin 1895, and must be closely related to *Nocardia tenuis* Castellani 1911, which belongs to the same genus, and as its colonies on agar are 'cerebriform' it may possibly be the same or related to *Streptothrix radiatus* and *S. cerebriformis*, both described from cases of keratitis by Namyslowski in 1909, as well as the more aerobic hyphal form of Silberschmidt's organism.

Unfortunately, a mistake was made, for Cohn was not aware that the name *Streptothrix* had already been given by Corda, in 1839, for another and quite different fungus, which is known as *Streptothrix fusca* Corda 1839, and which

is to be found in all works of any importance on systemic mycology. Therefore, as *Streptothrix* is not available, after many changes, the generic name has become *Cohnistreptothrix* Pinoy 1911, and to this genus Israel's human organism belongs. It differs from Bollinger's type of fungus in growing best anaerobically, in being difficult to cultivate, and in not producing arthrospores. Other allied organisms are *Cohnistreptothrix thibiergei* (Ravaut and Pinoy 1909), also found in Actinomycosis in man; *Streptothrix spitzi* Lignières 1903, found in cattle, is probably identical with *C. israeli*, as may be Doyen's *Streptothrix*; while *Nocardia carougeaui* Gougerot 1909, in juxta-articular nodules, and *Streptothrix cuniculi* Schmorl 1891, probably also belong to this genus, as well as the *Streptothrix* recently discovered in a liver abscess in America by Bloomfield and Bayne-Jones (1915), as we have consulted the authors upon this point, with which they are in agreement. Perhaps the bacillus described by Sawtschenko, in 1896, as the causal agent of a Pseudomycetomatous condition may also belong to this genus, and it is also possible that the *Coccobacillus pseudo-actinomycosis polymorphus* Berestneff 1898 may be the same as the chromogenic anaerobic streptothrix, obtained from human pus by Neschadimenko in 1908, and carefully described.

8. *C. israeli*. This organism appears to be of increasing importance in human pathology, for, according to Pinoy, it appears to affect man more often than *N. bovis*. It was first discovered in man, as mentioned above, by Wolff and Israel in Germany, and has since been found in thirteen cases in the United States by Wright. It has also been found in cattle by Lignières and Spitz (1904) in the Argentine, and by Pinoy (1913) in France.

It is composed of short and long rods, some of which show club-like swellings, while in old cultures spores which resemble cocci in appearance can be seen. It grows but poorly in the presence of air, but much better anaerobically at 37° C. on agar, on which it forms dew-like drops, which later become yellowish and generally remain discrete. In broth it forms a deposit of small scaly particles. It does not grow on gelatine at the room temperature of Europe, but egg cultures show typical branched filaments with club-like ends, which later break up into bacillary and coccid forms, but true arthrospores (i.e. resistant spores) are not produced. It forms granulation tumours when inoculated intraperitoneally into rabbits and guinea-pigs, after an interval of four to seven weeks. In these tumours typical actinomycotic grains can be found, containing branched filaments with clavate ends.

9. *C. thibiergei*. This fungus was discovered in 1909 by Ravaut and Pinoy in a case of Actinomycosis which produced generalised subcutaneous and intramuscular nodules in a man in France.

The nodules opened and discharged blood-tinged pus, in which the fungus was seen sometimes in isolated bacillary form and sometimes as very small white grains, which in the tissues might measure some 80 microns and be composed of a radiating mycelium with or without fine club forms. It grows well aërobically and anaërobically, but the former produces more bacillary and the latter more filamentous forms. The optimum temperature is about 37° to 38° C. It does not appear to be pathogenic for laboratory animals.

Actinobacillus. This curious Gram-negative bacillus-like organism, discovered by Lignières and Spitz (1904) in the Argentine, in cattle, has been shown by Griffith to be wide-spread in the world as a cause of Actinomycosis in cattle, in which the grains are very small and possess clubs. It grows well aërobically and anaërobically on various media, and does not liquefy gelatine or blood serum, but it gives rise to indol, and is pathogenic for many animals. It may also, in the future, be found to cause Actinomycosis in man, but we are only acquainted with one human infection, viz., that described in Paris, in 1911, by Ravaut and Pinoy, which occurred in a man from Argentine, causing otitis, mastoiditis and meningitis. It was obtained from the cerebro-spinal fluid, and on glucose peptone formed clubs, and this being so its name would become *Nocardia lignieresi* (Brumpt 1910). It may also have been the causal agent in the Pseudo-actinomycosis described by Cozzolino in 1900, but we have been unable to refer to this author's writings, and, therefore, leave this merely as a suggestion, and, at all events, at present it need not be classified with the organisms causing human Actinomycosis in the sense defined above.

After the description and classification of the organism which we have found here, it will be necessary to compare it with the nine fungi just described as causal agents in cases of Actinomycoses, and therefore we now turn to the description of the Sudanese Actinomycosis and its causal fungus.

THE SUDANESE ACTINOMYCOSIS

The specimen about to be described was in the form of a small roundish fibrous tumour situate on the dorsum of the foot, the skin of which was not affected, in an adult Sudanese man who came to the Khartoum Civil Hospital for treatment. There was no enlargement of the lymphatic glands, no sinuses and no discharge.

The growth was found to be lying in the subcutaneous tissue and was removed entire, and the patient made an excellent recovery, and, so far as is known up to the time of writing this paper and one year after the operation, there has been no recurrence.

The growth was cut into halves, from one of which the yellowish grains were removed for purposes of cultivation, while from the other half (fig. 1) sections were made.

PATHOLOGICAL HISTOLOGY

When fig. 1 is examined, it will be observed that it represents a section of the growth magnified one and a half times, and shows that for purposes of description it can be divided into two portions, viz. :—

1. A dense matrix.
2. A number of irregularly shaped darker bodies, 'the fungal masses,' embedded in the matrix.

The Matrix. When the matrix (fig. 13) is studied by the aid of higher magnifications, it will be seen to be composed of white fibrous connective tissue containing a large number of connective tissue corpuscles, and here and there a blood vessel or a small group of blood vessels which may or may not be associated with a collection of cells (fig. 13), and, in addition, lymph spaces and small collections of fat cells mostly associated with the blood vessels (fig. 13). When these vessels are studied more carefully, some will be observed to be more or less normal, while others show signs of periarteritis (fig. 13) or endarteritis (fig. 14) of varying degree, which produce diminution and even occlusion of the lumen.

Connected with many of these vessels, and often more or less surrounding them, lie dense masses of cells (fig. 13), which when carefully studied (fig. 15) appear to be all mononuclear. They are not all of the same category, however, for some, judging by their nuclei, appear to be derived from the endothelial cells of the vessels. Another type of cells is characterised by a darker staining nucleus, and appearing when cut in certain directions as though it possessed very little cytoplasm, but, when seen more correctly, has a relatively fair quantity of cytoplasm in proportion to the size of the nucleus. The nucleus being placed excentrically, and the cytoplasm being non-granular and not eosinophile, this cell agrees with Unna's description of a healthy *Plasma Cell* as seen in Actinomycosis.

A third type of cell shows a large vesicular nucleus situate excentrically in a relatively large quantity of cytoplasm, which is either eosinophile or contains eosinophile granules, and corresponds exactly with Unna's description of degenerating plasma cells as seen in Actinomycosis.

Fungal Masses. The darker irregular bodies seen embedded in the matrix in fig. 1, if examined by the aid of higher magnification,

can be seen to consist of fibrous tissue and cells surrounding a portion of the fungus (fig. 12), and have therefore, for purposes of distinction, been termed '*Fungal Masses*.'

When a typical fungal mass is examined (fig. 12) by means of a moderately high magnification, it can be seen to be composed of several distinct areas which, working from the fibrous tissue matrix towards the fungus, lie in the following order:—

1. *The Fibrous Sheath.* This is continuous with the fibrous tissue forming the matrix of the whole growth, as already described.
2. *The Fibro-cellular Layers.* Directly under the dense fibrous tissue there lies a thicker or thinner area composed of loose fibrous tissue, containing in its meshes cells and thin-walled vessels; this area may be termed the fibro-cellular layers.
3. *The Cellular Sheath.* Internal to the fibro-cellular layers comes a mass of cells which may be called the cellular sheath.
4. *The Grain.* Situate in the cellular sheath there lies a more or less distinctly or indistinctly striated body, of varying shape, and often with irregular edges, which is the grain, and is composed of the fungus and its surrounding matrix.

It is proposed to postpone the study of the grain until we discuss the aetiology of the growth, but a few words are necessary with regard to the areas.

Fibrous Sheath. When the fibrous connective tissue forming the matrix is examined, in the vicinity of a fungal mass, it will be observed to show collections of cells at intervals.

Fibro-cellular Layers. If figs. 12 and 18 are examined, it will be seen that these layers are composed of loose fibrous tissue, holding in its meshes plasma cells, healthy and degenerating polymorphonuclear cells, giant cells and blood vessels.

With regard to the giant cells, they may be seen to contain fungal masses (fig. 19), or these may be observed (fig. 16) escaping therefrom, or the giant cells may be remarked to be separated from the fungal mass by a little distance (fig. 17) and to be damaged, while polymorphonuclear cells lie near the fungus, and the adjacent layers of the fibro-cellular tissue may be observed to be arranging themselves circularly (fig. 17) so as to circumscribe the new fungal growth, and so to commence the formation of a new fungal mass.

When two fungal masses lie in close approximation to one another without the intervention of dense fibrous tissue, it will be observed that small areas of the fibro-cellular layers adjoining the two masses show signs of granular degeneration.

Another interesting feature, but by no means confined to the fungal masses, is the presence of cells containing one or several, small or large, rounded eosinophile globules (fig. 2). These were called Fuchsin or Russell bodies by Kanthack, and Botryomycotic bodies by Archibald (1911), who published some excellent illustrations thereof in Plates XV and XVI of the Medical Volume of the Fourth Report of these Laboratories. They are depicted in fig. 2, and appear to be a product of the fungus as we have frequently seen them in Nocardial infections lying in cells at a distance from the fungus, in which case they are a great aid in diagnosis as indicating the probable presence of a fungus somewhere. We have also seen them in masses cut longitudinally and lying in lymph spaces. We have observed them, but much more rarely, in Black Maduromycosis. They have been recorded by all workers at Actinomycosis and Maduromycosis since the days of Kanthack, and appear to us to be probably the same material as that forming the club-like dilatation of the extremities of the hyphae in *N. bovis* and other Nocardias, and that they may possibly be a protective substance excreted by the fungus which only under certain conditions consolidates into the eosinophile form demonstrated in fig. 13 and into the clubs of certain species of Nocardia.

The Cellular Sheath. All our observations tend to support Brumpt's view that primarily the fungus is enclosed in a cell which in the younger fungal areas near the older area is always multinuclear (fig. 19).

Further, in the present specimen, there can be no doubt that the fungus is not destroyed by the giant cell, but on the contrary, as shown in fig. 16, grows and escapes therefrom and starts life as a little fungal mass of its own (fig. 17), in which instance the polymorphonuclear leucocytes now appear upon the scene, and the fibro-cellular coat begins to circumscribe the cells and the fungus, while the damaged remains of the giant cell are seen retiring towards the periphery (fig. 17).

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Later, the mononuclear cells mentioned above appear, and these

various cells, together with detritus from the destruction of similar cells situate in a granular network, form the cellular sheath of the grain, as shown in our specimens. This description, although varying in detail, does not differ materially from a composite picture such as can be derived by a study of the writings of Carter, Acland, Kanthack, Boyce and Surveyor, Unna, Schlegel, Foulerton, Brumpt, and other authors who have studied the reaction of the body against different species of the genus *Nocardia*.

An analysis of its findings will be observed to support the view as to the pathogenesis of the structures found in Actinomycosis set forth by Brumpt in 1905-1906.

AETIOLOGY

The removal intact of the small growth completely cured the condition, therefore the aetiological factor must have been contained therein.

The only feature of the growth which cannot be attributed to the reaction of the body is the grain, and as this is composed of a fungus and its products, and as all the steps of the bodily reaction can be traced seriatim to be directed against this fungus, and against it alone, it becomes sufficiently manifest that this organism is the cause of the disease, even though the final proof of the reproduction of the disease by inoculations of cultures into animals is wanting, as all our experiments have been negative.

The points requiring investigation with regard to the aetiology of the Sudan Mycetoma may be classified into:—

1. The histology and mycology of the grain.
2. The cultivation of the fungus.
3. The mycology of the cultures.
4. The classification of the fungus.
5. Animal inoculations.
6. Comparison with the fungi known to cause other forms of Actinomycosis.

1. *The Grain.* As the skin covering the tumour was unbroken, and there was no discharge, grains could only be obtained by removal from the portion of the specimen set aside for this purpose.

A grain so obtained, and magnified twenty times, is depicted in

fig. 3. It was a soft, smooth, lobulated, yellowish body with a slight tinge of an orange hue mixed with the yellow, and measured about 0.5 millimetre in diameter. To be more exact, it resembled Ridgway's Color Standard, Plate III, 17 O-Y, f, Pale Orange-Yellow.

When a portion was flattened out between a slide and cover-glass, and then examined by means of a fairly high magnification, it was seen that the grain was composed of typical nocardial bacillus-like hyphae (fig. 6) embedded in a more or less homogeneous matrix. The width of a given hypha was less than one micron, and no definite thick hyphal wall, such as is easily discerned in a fungus like *Aspergillus*, could be detected, nor could any septa be seen.

On examining sections stained by haematoxylin and eosin, it is observed that the fungal matrix is stained by the haematoxylin, while the hyphae remain as clear, radiating, unstained branched spaces. The margin of the grain does not possess a sheath (fig. 18), and may be even or may be irregular, having well defined processes jutting into the cellular sheath, but is in any case closely attached to polymorphonuclear cells, or in much younger grains to these and giant cells. The hyphal filaments are well coloured by the Gram-Weigert method, and are then seen to be branching or bacillary-like in appearance and to contain homogeneous or beaded contents. No clubs or club-like ends could be detected, on the contrary the ends were thin and rounded.

It is, therefore, evident that the grain is composed of branching, exceedingly fine hyphae, not markedly septate and without the usual thick and well defined walls of the typical fungal hypha, and containing either homogeneous protoplasm or this with darker staining bodies at intervals, and embedded in a more or less homogeneous matrix.

2. *Cultivation.* The fungus obtained from the grains described in the preceding section grows fairly well at 22°C., best at 30°C., moderately well at 37°C., and not at all at 60°C. under aërobic conditions (fig. 8). It also grows well under anaërobic conditions (fig. 9), but the white efflorescence is more marked than upon aërobic growths. The optimum medium and temperature appear to be Blood Serum at 30°C., i.e. upon an alkaline medium. It appears to prefer alkalinity, as potato has to be rendered slightly alkaline

before it will grow well. It is Gram-positive, but not acid-fast, as tested by acid decolorization. Its cultures have no distinct odour, and are usually warm buff in colour (i.e. Ridgway's Standards, Plate XV, 17 O-Y, d) when well developed and free from efflorescence.

In Peptone Broth at 37° C., it forms numerous white non-cohering flocculi which sink to the bottom of the tube, while the medium remains quite clear and unpigmented, and there is no growth on the surface.

In Glucose Peptone, at 37° C., the growth resembles that in Peptone Broth.

In Litmus Milk, at 30° C. and at 37° C., it grows well, but it neither acidifies nor clots this medium at any time nor does it form a surface growth, but it appears to increase the alkalinity of the medium.

In Nutrient Gelatine, in stab cultures incubated at 22° C., it forms a surface growth in the form of small rounded light buff colonies, while along the depth of the stab for its entire length it gives rise to numerous minute colonies at the end of nine days. These growths gradually become smaller and smaller as the distance from the surface into the depth of the gelatine is increased. It continues to grow in gelatine long after the eighth day after inoculation, but it never causes liquefaction or pigmentation of the medium.

On Agar-Agar, if the medium is alkaline, it forms the typical warm buff-coloured convoluted growth in forty-eight hours at 37° C., and is often surrounded by secondary younger colonies. At 30° C., in the same time, the convoluted growth is more marked, but is much paler in colour, while there are but few secondary isolated young colonies (fig. 28). It is not adherent to, nor does it pigment the medium; it produces no smell. If the agar is acid no growth takes place.

On Clear (not Sabouraud's) Maltose Agar, at 30° C. and 37° C., it gives rise to raised, rounded or oval, corrugated, moist light buff coloured colonies (fig. 5), which when young have a radiating fringe of white rays, but when old possess only a glistening light whitish margin level with the surface of the medium into which the growth does not penetrate and which does not become pigmented.

On Glycerine Agar, at 37° C., it forms a raised, translucent, moist, warm buff coloured growth, which, however, is not as luxurious as upon maltose agar. A given colony upon this medium has clear cut edges or a whitish margin lying nearly level with the surface, and does not sink into nor pigment the medium.

On Glucose Agar (fig. 29) it produces in three days at 30° C. a warm buff coloured growth which is markedly convoluted, somewhat resembling the convolutions of the brain after the removal of the pia mater. The medium is not pigmented.

When grown on *Sabouraud's Maltose Agar* it produced a typical growth, which is depicted in fig. 23, but it was more radiated than convoluted, and had well marked white efflorescence.

On Sabouraud's Preservative Medium it grows well under aërobic (figs. 4 and 8) but not so typically under anaërobic conditions (fig. 9) at 30° C.

On Inspissated Ox Blood Serum it grows well, forming in seven days at 30° C. light buff coloured, raised, coiled colonies (fig. 10), which, after twelve days' growth at the same temperature, begin to liquefy and clear the medium (fig. 11), which they do not pigment.

On Potato it grows extremely well at 30° C. to 34° C., forming a light buff coloured, raised, convoluted, moist growth, on which a white efflorescence begins to form after nine days. At 37° C., in about forty-eight hours, it produces a typical convoluted growth, which in four days becomes surrounded by many small colonies showing a whitish efflorescence (fig. 7). The medium is neither eroded nor pigmented.

On Carrot it grows well, giving rise to a light buff coloured, raised, corrugated, moist growth, which neither erodes nor pigments the medium.

In Starch Peptone Medium it grows well, but shows no diastatic action after nine days' incubation at 37° C., a control tube being used.

In Sugar Media there was a good growth of the puff ball variety, but no formation of acid or gas as tested qualitatively. The sugars used were glucose, lactose, maltose, saccharose, raffinose, salicin and mannitol.

3. *Mycology.* Whether growths or grains are examined, the outstanding feature of the mycology is that the fungus consists of a

branching mycelium composed of exceedingly thin hyphae, generally less than one micron in diameter, without the usual thick hyphal wall which one is accustomed to associate with fungal filaments. These hyphae may contain homogeneous Gram-positive cytoplasm, but more commonly they exhibit a beaded appearance, with areas of intense staining separated by non-coloured (fig. 22) or almost colourless intervals.

The hyphal threads may be of considerable length, as, for example, the one depicted in fig. 20, but they fail to show the septa so commonly met with in fungal hyphae. When old, the cytoplasm of a hypha breaks into fine granules (fig. 25), which, becoming absorbed, leave an empty sheath, which (fig. 24) ceases to retain Gram's stain and becomes tinged with the counter stain. This sheath breaks (fig. 24) and disappears, and now the areas retaining the stain become separated as longer or shorter rods (fig. 21), which resemble bacilli, especially the diphtheria bacillus, in appearance. As these rods can, without doubt, give rise to new mycelial threads, they appear to us to be analogous, though perhaps not homologous with *Thallospores*.

Hyphal filaments, however, generally show branching which is not dichotomous but irregular in arrangement (fig. 21), and after a certain amount of growth they often give rise to hyphae which produce rows or chains of spores (fig. 26), which form the whitish efflorescence often seen on growths. These spores being Gram-positive, rounded bodies, about a micron in breadth, closely resemble cocci, and, therefore, an old growth with its beaded bacillary forms and its rounded spores resembles most closely a collection of bacilli, micrococci and streptococci, and for such the fungus is sometimes mistaken even at the present.

The *Thallospore*-like hyphae may be the agency by which the numerous small colonies form round a parent colony, as seen in fig. 7, while the rounded spores appear to us to represent arthrospores, and presumably help to keep the fungus in existence in times of difficulty. They are shown sprouting in fig. 27.

Beside these two kinds of reproduction we have failed to find any reproductive mechanism, and the life cycle from the arthrospore giving rise to the hypha, which breaks into segments and forms a bacilliform mycelium on which the streptococcus-like chain

spores arise, corresponds exactly with the admirable descriptions of the life cycle of a typical parasitic *Nocardia*, as given and fully illustrated by Foulerton, and need not detain us here, and, therefore, we will pass on to consider the exceedingly difficult problem of the classification of this fungus, which appears to represent in itself the spore types of *Thallospore* and *Arthrospore*.

4. *Classification.* As the fungus in question does not possess known sexual cells, even though its hyphae do not exhibit the usual septa, it must be classified among Schröter's *Eumycetes*, and not among De Bary's *Phycomycetes*.

As its spores are not situated in asci or basidia, it belongs to Fuckel's class of the *Fungi Imperfecti*, and as no accessory fructifications in the form of open or closed receptacles have been observed in any of our cultures, it must belong to the sub-class named *Hyphales* Vuillemin 1910, which is divided into four orders according to the following scheme:—

A. Mycelium composed of fine bacilliform hyphae, usually one micron or less in diameter, with a thickened hyphal wall and septa	Order 1 <i>Microsiphonales</i> Vuillemin 1912
B. Mycelium composed of hyphae, usually greater than one micron in diameter, and usually with a thickened hyphal wall and septa:—	Order 2 <i>Thallosporales</i>
I. Reproduction by thallospores	Vuillemin 1910
II. Reproduction by hemispores	Order 3 <i>Hemisporales</i> Vuillemin 1910
III. Reproduction by conidia	Order 4 <i>Conidiosporales</i> Vuillemin 1910

A consideration of this table shows quite clearly that the fungus which we are considering belongs to Vuillemin's order *Microsiphonales*, which contains at present only two genera, which are distinguished as follows:—

A. Grows easily aërobically, and produces arthrospores	Genus 1 <i>Nocardia</i> De Toni and Trevisan 1889
B. Grows best anaërobically, but can grow aërobically, usually difficult to cultivate, and does not produce arthrospores	Genus 2 <i>Cohnistreptothrix</i> Pinoy 1911

Again there is no difficulty as to the classification, as the fungus which we are studying obviously agrees with the definition given above for the genus *Nocardia* De Toni and Trevisan 1889, but now the difficulty of the specific determination begins, and a very real and serious difficulty it is, and in attempting to make order out of the chaos into which the species of the genus have got, we have availed ourselves of Foulerton's broad lines of classification which, in our opinion, are of great value. It would merely weary the reader if we recorded the difficulties which we have met with in this task, or if we even gave the reasons for the classification which we bring forward. For our present purposes, it will suffice if we give the mere outlines of our results.

With Foulerton (1905-1912) we divide the species of the genus *Nocardia* into three *sections*, as follows:—

- | | |
|--|--|
| A. Habitat.—Soil, can be found in air or water ... | Section 1
<i>Saprophytica</i>
Foulerton 1910 |
| B. Habitat.—Plants or animals | Section 2
<i>Parasitica</i>
Foulerton 1910 |
| C. Habitat.—Soils, plants or animals, but imperfectly described | Section 3
<i>Incertae sedis</i> |

As our parasite has only so far been found in man, we may, for the time being, confine our attention to the second section to which it obviously belongs, though of course it might be a saprophytic species which had become parasitic in and pathogenic to man, but this question we will leave till later.

The Parasitic Section we have classified into three sub-sections, as follows:—

TABLE I

Diagnosis of the Sub-sections of the Section *Nocardia parasitica*

No.	Test	Sub-section 1 Majora	Sub-section 2 Minora	Sub-section 3 Brevis
1	Cultivation at 22° C. and 37° C.	Easy	Not difficult	Difficult at 37° C. Usually nil at 22° C.
2	Growth	Spreading	Circumscribed	Slight
3	Efflorescence	Bright chalky	Dull powdery	Usually absent
4	Hyphal branching	Well marked	Poorly marked	Rare, hyphae often bacilliform
5	Acid fast species	Rare	Common	Rare
6	Odour of cultures	Earthy or mouldy	Absent or faintly as 1	Sometimes faeculent
7	Liquefaction of gelatine and blood serum	Often present	Rare, and usually only one liquefied	Often very slight indications
8	Potato	Growth	Usually growth	Often no growth
9	Diastatic action	Often present	Usually absent	Not known

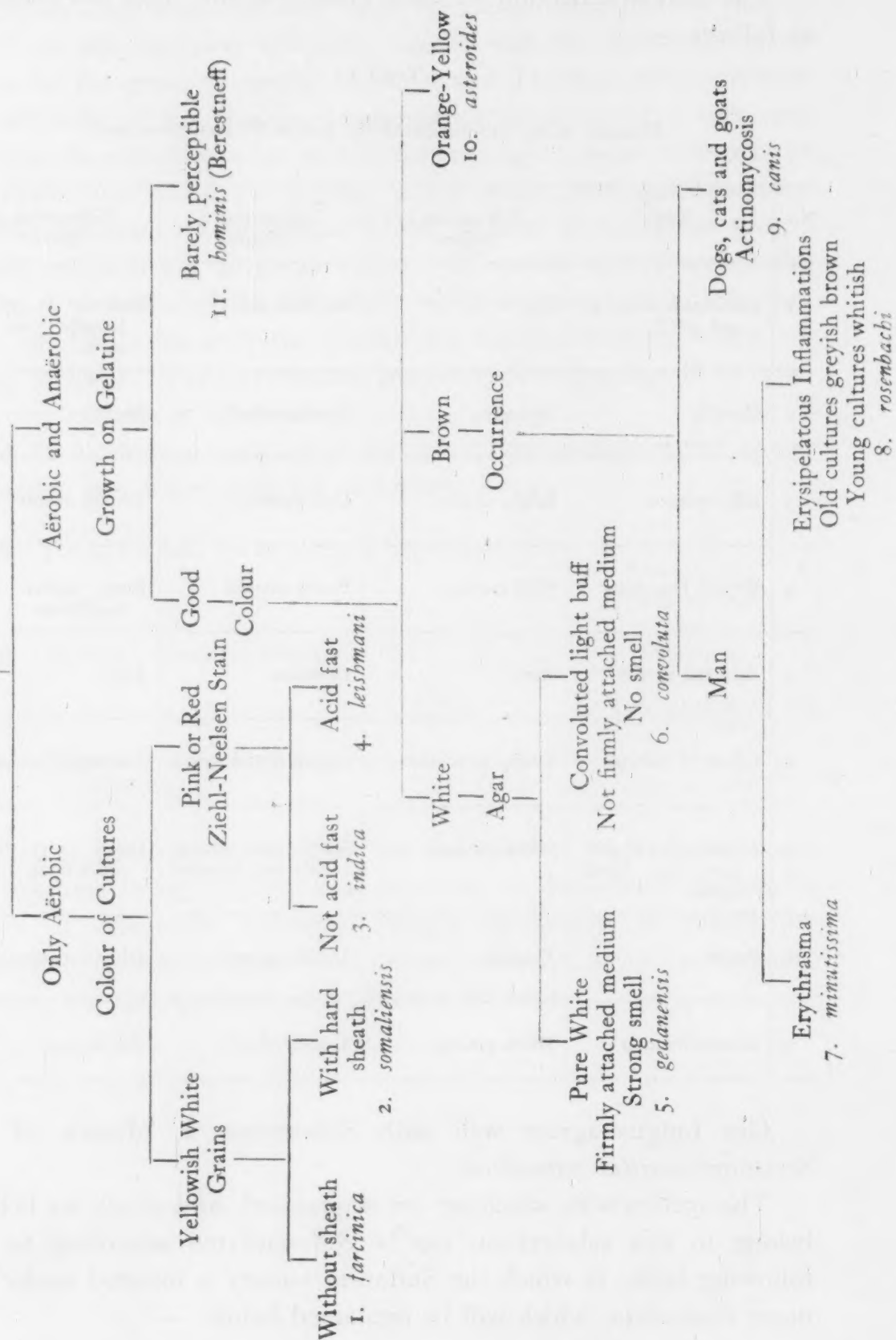
Our fungus agrees well with Sub-section 2, Minora, of the Section *Nocardia parasitica*.

The species with which we are acquainted, and which we believe belong to this sub-section, can be differentiated according to the following table, in which the Sudanese variety is inserted under the name *Convoluta*, which will be explained below:—

TABLE II

Diagnostic Table of the Species of Section *Paratitica*, Sub-section *Minora*

Primary Growth at 22° C. and 37° C.



LIST I

Synonyms of the species tabulated in Table II:

1. *Nocardia farcinica* Trevisan 1889
Bacillus du Farcin Nocard 1888
Streptothrix farcinica Rossi-Doria 1891
2. *Nocardia somaliensis* (Brumpt 1906)
Indiella somaliensis Brumpt 1906
Indiellopsis somaliensis (Brumpt 1913)
Discomyces somaliensis Brumpt 1913
? Ballingall's Disease
3. *Nocardia indica* (Kanthack 1893)
Oospora indica Kanthack 1893
Streptothrix madurae Vincent 1894
Discomyces madurae Vincent 1895
Nocardia madurae R. Blanchard 1895
Micrococcus pelletieri Laveran 1906
Oospora pelletieri Thiroux and Pelletier 1912
Nocardia pelletieri Pinoy 1912
Nocardia rivierei Verdun 1912 ?
4. *Nocardia leishmani* new name
New acid fast *Streptothrix* pathogenic to Man and Animals described by Birt and Leishman in 1902
5. *Nocardia gedanensis* (Scheele and Petruschky 1897)
Streptothrix gedanensis I Scheele and Petruschky 1897
6. *Nocardia convoluta* new species
7. *Nocardia minutissima* (Burchardt 1859)
Microsporum minutissimum Burchardt 1859
Trichothecium J. Neumann 1868
Microsporon gracile Balzer 1883
Sporotrichum minutissimum Saccardo 1886
Microsporoides minutissimus Neveu-Lemaire 1908
Discomyces minutissimus Brumpt 1910
Oospora minutissima Ridet 1911
Nocardia minutissima Verdun 1912
8. *Nocardia rosenbachii* (Kruse 1896)
Streptothrix rosenbachii Kruse 1896
9. *Nocardia canis* (Rabe 1888)
Cladothrix canis Rabe 1888
Streptothrix caprae Silberschmidt 1899
10. *Nocardia asteroides* (Eppinger 1890)
Cladothrix asteroides Eppinger 1890
Streptothrix eppingeri Rossi-Doria 1891
Oospora asteroides Sauvageau and Radais 1892
Nocardia asteroides R. Blanchard 1895
Streptothrix hominis Sabrazès and Rivière 1895
Actinomyces asteroides MacCallum 1902
Discomyces asteroides Brumpt 1906
Streptothrix freeri Musgrave and Clegg 1907
Discomyces brasiliensis Lindenberg 1909
The organisms described by Ferré and Faguet, by McCallum, by Schabad probably belong to this species.

II. *Nocardia hominis* (Berestneff 1897)

- Nec *Actinomyces hominis* Bostroem synonym of *N. bovis*
- Nec *Actinomyces hominis* Affanasieff = *N. bovis*
- Nec *Actinomyces hominis* Wolff and Israel = *N. israeli*
- Nec *Streptothrix hominis* Sabrazès and Rivière = *N. asteroides*
- Nec *Streptothrix hominis* Hayo Bruns 1899
- Nec *Streptothrix hominis* Foulerton 1902
- Nec *Streptothrix hominis* II Foulerton 1910
- Nec *Streptothrix hominis* III Foulerton 1905 = *N. bovis*
- Nec *Streptothrix hominis* IV Foulerton 1910 = *N. bovis*
- Nec *Streptothrix hominis* III Foulerton 1910

As there is so much confusion with regard to the specific name '*hominis*,' we propose that:—

- S. hominis* Bruns be changed to *Nocardia bruni*
- S. hominis* Foulerton be changed to *Nocardia foulertoni*
- S. hominis* II Foulerton be changed to *Nocardia londinensis*
- S. hominis* III Foulerton be changed to *Nocardia appendicis*

This table shows that the Sudan fungus (called in the table *Convoluta*) can be differentiated from its allies, but it may be said that we have not separated it sufficiently from the various species belonging to the Sub-sections *Brevis* and *Majora*, and, in order to meet this requirement, we give an Appendix of Diagnostic Tables and Lists of Synonyms at the end of this paper, in which Tables III and IV and Lists II and III will enable the reader to understand our views as to the differential diagnosis and synonyms of the various species, belonging to these two sub-sections, with which we are acquainted.

In order to show that our Sudanese fungus is not a known saprophytic species, we have followed Foulerton and divided the Section *Saprophytica* into two Sub-sections, viz., *Minora* and *Majora*, with the same characters as for the Section *Parasitica*, excluding only the acid-fast character.

We have further differentiated the species belonging to these two sub-sections in Diagnostic Tables VI and VII of the Appendix just mentioned, and we give the synonyms in Lists IV and V of the same Appendix.

A study of these tables will show that the Sudanese fungus is not similar to any species contained therein.

In order to complete the differentiation, we also provided in List VI the names of a few species of *Nocardia* belonging to Section 3, *Incertae Sedis*, while in Table VIII and List VII we give

the differentiation and the synonyms of the species of the genus *Cohnistreptothrix*.

These tables and lists do not profess to be final, or even full, but merely to contain such species as we have been able to find in the limited literature at our disposal in Khartoum, and very many more must exist scattered in Medical, Veterinary, Agricultural and Botanical writings.

They are intended to show that, as far as we know, the species of *Nocardia* found in the Sudanese Actinomycosis is new to human parasitology, and also new to science, and, therefore, we name it *Nocardia convoluta* Chalmers and Christopherson 1916, and derive the name from the latin '*convolutus*,' signifying twisted, because of its peculiar growth on potato, blood serum and the agars, as depicted in figs. 7, 10, 11, 28 and 29, and we define it as follows:—

'*Nocardia*. Gram-positive but not acid-fast and without club formations, found parasitic in man, easy of cultivation and growing aërobically and anërobically at 22° C. and 37° C., with a marked preference for alkaline media, and with the production of good but limited growths on the different agars, and the same at first on blood serum and potato, on which, however, it becomes more profuse later. Not liquefying gelatine, but causing liquefaction of inspissated ox blood serum, and without diastatic action. Colonies usually somewhat translucent when young, and of a light to warm buff colour (Ridgway's Plate XV, 17, O-Y, f or d), and either convoluted or having the appearance of a jelly turned out of a mould, but later developing a whitish powdery efflorescence, without distinct odour, and never pigmenting the medium on which it is grown and not fermenting or peptonising milk. Non-pathogenic for monkeys and other laboratory animals.'

5. *Inoculation in Animals*. All attempts to infect monkeys, white rats, gerbils, rabbits and pigeons by various methods of inoculation have, so far, failed in our hands.

6. *Comparisons*. In the historical part of this paper we invited attention to seven species of *Nocardia* and two species of *Cohnistreptothrix* known to cause Actinomycosis, and with these our Sudanese variety must be compared, but, before so doing, we must invite attention to the *Black Actinomycosis* of Babès and

Mironescu, of which the causal fungus has not been cultivated, and, therefore, its generic classification is doubtful.

In the year 1888, Babès, in Roumania, met with a case resembling an Actinomycosis, in that the pus, escaping via a fistula from a deep abscess, showed small black grains with actinomycotic clubs. The pus also contained pyogenic cocci.

In 1910, he and Mironescu met with a second case of a similar nature, also in Roumania. In this case a retro-bulbar abscess opened by means of a fistula in the upper eyelid. Eventually the eye became diseased and had to be removed, an abscess formed in the brain, and the man died. In the pus from these abscesses they found pyococci and black grains, which latter were curious in that they were not due solely to the contained fungus but were formed by the connective tissue being changed into black masses.

The fungus was composed of thick Gram-negative hyphae, measuring two microns in diameter and *falsely* branched at acute or right angles. They appear to have had a distinct membrane but not a thick hyphal wall, and septa are not described or illustrated.

The authors lay stress upon the thickness of the hyphae and the false branching as distinguishing this fungus from a *Nocardia* (*Streptothrix*). All attempts to grow this causal organism or to infect animals were negative, and, therefore, they are unable to classify the fungus, which they doubtfully think may be *Cladothrix*.

When the illustrations are examined, the fungus is seen to resemble a *Nocardia* with thick hyphae, especially as true branching is depicted in fig. 3, but, be that as it may, the descriptions given by the authors agree with our definitions of 'Mycetoma,' 'Granum' and 'Actinomycosis,' and, therefore, though the systemic position of the fungus is doubtful, still the disease is an Actinomycosis with black grains, and as such must be distinguished from the Sudanese variety.

A method of differentiation of these various Actinomycoses is set forth in the following Table:—

A. Grains black, fungus not cultivated	<i>Babès and Mironescu's Actinomycosis</i>
B. Grains white, yellow, reddish yellow, yellowish red or red, fungus cultivated:—	
I. Cultivation difficult, grow best anaërobically, arthrospores absent	Genus 1 <i>Cohnistreptothrix</i>
a. Clubs present:—	
1. Grains yellow	1. <i>israeli</i>
2. Grains very small and white	2. <i>thibiergei</i>
II. Cultivation easy, grow best aërobically, arthrospores present	Genus 2 <i>Nocardia</i>
a. Clubs present	3. <i>bovis</i>
b. Clubs absent:—	
1. Grains surrounded by a hard sheath, insoluble in liquor potassae and eau de Javelle	4. <i>somaliensis</i>
2. Grains without such a sheath:—	
Gi. Growth on gelatine absent	5. <i>krausei</i>
Gii. Growth on gelatine present:—	
M. Inspissated blood serum liquefied:—	
x. Pathogenic for laboratory animals, growth on potato white, medium becomes greenish in colour	6. <i>garteni</i>
y. Non-pathogenic for laboratory animals, growth on potato yellowish or buff colour, medium unchanged:—	
ri. Gelatine liquefied, and growths not convoluted	7. <i>liquefaciens</i>
rii. Gelatine not liquefied, and growths markedly convoluted	8. <i>convoluta</i>
N. Inspissated blood serum not liquefied:—	
x. Growths yellowish orange to brick red	9. <i>asteroides</i>
y. Growths at first whitish, later pink	10. <i>indica</i>

By this Table it will be observed that *N. liquefaciens* and *N. convoluta* come into close relationship, but in addition to the former belonging to the *Majora* and the latter to the *Minora* sub-

sections of the *Parasitic Section*, the following differences may be noted:—

Reaction	<i>N. liquefaciens</i>	<i>N. convoluta</i>	Comparison
Conditions of growth	Obligatory aërobe	Facultative anaërobe	Different
Gelatine	Liquefaction begins 4th or 5th day	Not liquefied	Different
Colour of growths	Yellow becoming red-dish yellow	Light to warm buff	Different
Medium	In old cultures tinted, dark yellow	Not tinted	Different
Potato	Small yellow colonies in two days	Convolutated growth in two days	Different
Amount of growth	Considerable and not restricted	Usually restricted	Different

Therefore, we conclude that *N. convoluta* can be easily distinguished from *N. liquefaciens*, and that it gives rise to a separate form of human Actinomycosis, of which the following table contains a list of the varieties of this disease as known to us:—

A. WITH WHITE, YELLOW OR RED GRAINS

1. *Carter's Actinomycosis* caused by *Nocardia indica* (Kanthack 1893)
2. *Acland's Actinomycosis* caused by *Nocardia bovis* (Harz 1877)
3. *Israel's Actinomycosis* caused by *Cohnistrepthothrix israeli* (Kruse 1896)
4. *Eppinger's Actinomycosis* caused by *Nocardia asteroides* (Eppinger 1890)
5. *Hesse's Actinomycosis* caused by *Nocardia liquefaciens* (Hesse 1892)
6. *Krause's Actinomycosis* caused by *Nocardia krausei* (Chester 1901)
7. *Bouffard's Actinomycosis* caused by *Nocardia somaliensis* (Brumpt 1906)
8. *Garten's Actinomycosis* caused by *Nocardia garteni* (Brumpt 1908)

9. Ravaut and Pinoy's Actinomycosis caused by *Cohnistreptothrix thibiergei* Ravaut and Pinoy 1909
10. Chalmers and Christopherson's Actinomycosis caused by *Nocardia convoluta* Chalmers and Christopherson 1916

B. WITH BLACK GRAINS

11. Babès and Mironescu's Actinomycosis with unclassified fungus

SUMMARY

We believe that we have found a new form of Actinomycosis in man in the Sudan, and that the causal fungus is new in man and also new to science, and, therefore, we name it *Nocardia convoluta*, which makes the number of different varieties of the Actinomycotic form of Mycetomas known to exist in man eleven in all.

Diagnosis can only be effected by finding and cultivating the grain, associated with a study of its mycology and that of the cultures therefrom, together with a comparison of the results so obtained with the tables given above.

The treatment which cured this condition was the complete removal of the whole growth, i.e. the removal of all the fungal elements.

ACKNOWLEDGMENTS

It gives us much pleasure to acknowledge the kindness received from Dr. Asland, and especially from Dr. Foulerton, during the preparation of this paper. The latter most generously supplied us with cultures wherewith to make comparisons with our fungus.

KHARTOUM,

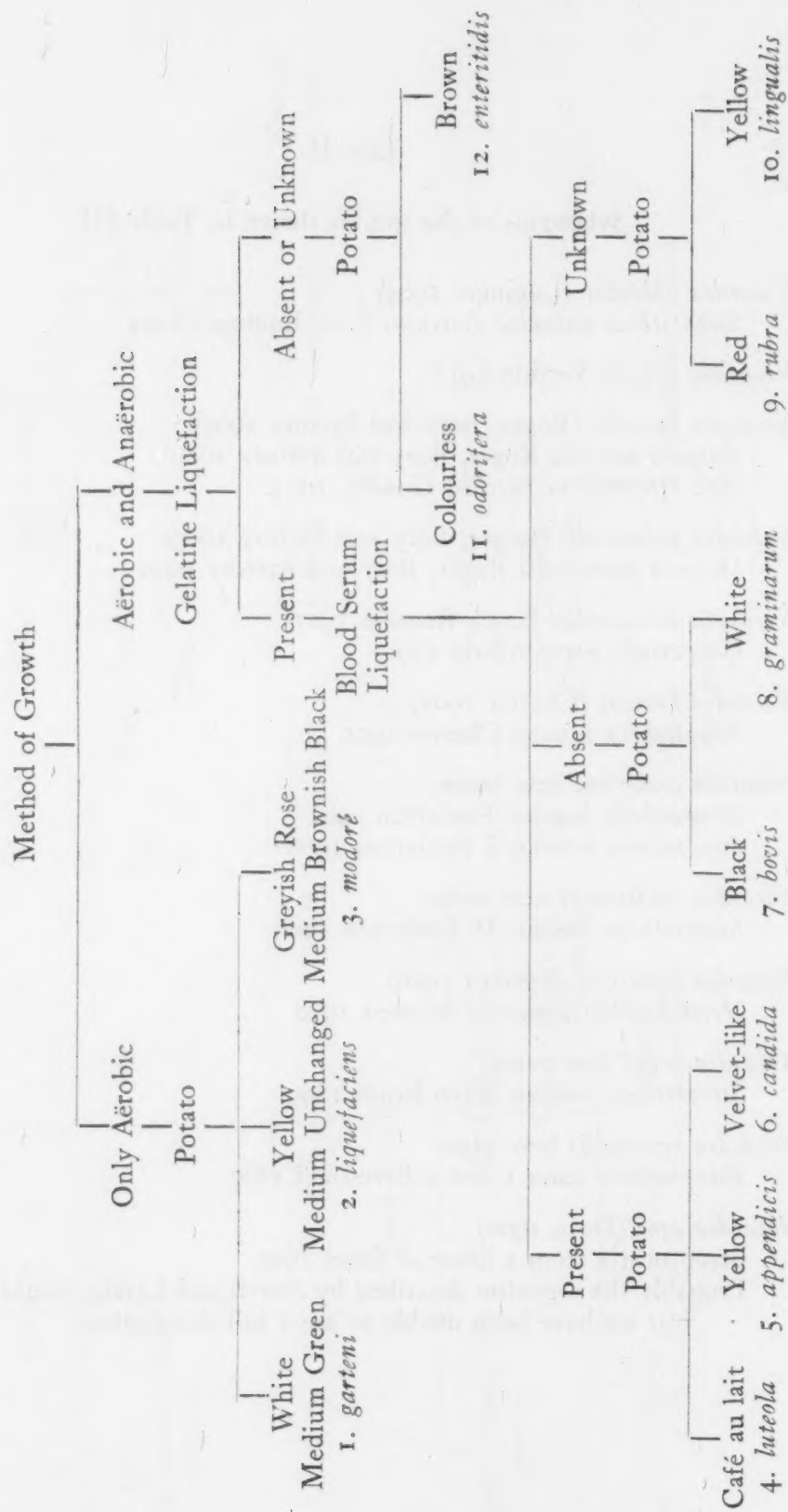
June 1st, 1916.

LIST II

Synonyms of the species shown in Table III

1. *Nocardia valvulae* (Luginger 1904)
Streptothrix valvulae destruens bovis Luginger 1904
2. *Nocardia ponceti* Verdun 1912
3. *Nocardia buccalis* (Roger, Bory and Sartory 1909)
Oospora buccalis Roger, Bory and Sartory 1909
Nec Streptothrix buccalis Goadby 1903
4. *Nocardia pulmonalis* (Roger, Bory and Sartory 1909)
Oospora pulmonalis Roger, Bory and Sartory 1909
5. *Nocardia dassonvillei* Brocq-Rousseu 1907
Gasparini's *Streptothrix* 1890
6. *Nocardia krausei* (Chester 1901)
Streptothrix krausei Chester 1901
7. *Nocardia foulertoni* new name
Streptothrix hominis Foulerton 1902
Streptothrix hominis I Foulerton 1906
8. *Nocardia londinensis* new name
Streptothrix hominis II Foulerton 1906
9. *Nocardia lignieresi* (Brumpt 1910)
Actinobacillus lignieresi Brumpt 1910
10. *Nocardia bruni* new name
Streptothrix hominis Hayo Bruns 1899
11. *Nocardia berestneffi* new name
Streptothrix cases 1 and 2 Berestneff 1897
12. *Nocardia equi* (Dean 1900)
Streptothrix from a horse of Dean 1900
Probably the organism described by Norris and Larkin should come here,
but we have been unable to see a full description.

TABLE IV

Diagnostic Table of the species of the Section *Parasitica*, Sub-section *Majora*

LIST III

Synonyms of the species tabulated in Table IV

1. *Nocardia garteni* (Brumpt 1910)
Cladothrix liquefaciens II Garten 1895
Discomyces garteni Brumpt 1910
2. *Nocardia liquefaciens* (Hesse 1892)
Cladothrix liquefaciens Hesse 1892
Streptothrix liquefaciens (Hesse 1892)
Streptothrix buccalis Goadby 1903 nec Roger, Bory and Sartory 1909
3. *Nocardia modoré* Thiry 1897
Cladothrix modoré Thiry 1897
Cladothrix polychromes Thiry 1897
Actinomyces rubidaureus Lachner-Sandoval 1898
4. *Nocardia luteola* (Foulerton 1910)
Streptothrix luteola Foulerton 1910
5. *Nocardia appendicis* new name
Streptothrix hominis III Foulerton 1910
Streptothrix hominis IV Foulerton 1906
6. *Nocardia candida* (Petruschky 1898)
Streptothrix candida Petruschky 1898
Streptothrix gedanensis II Petruschky 1898
Streptothrix lathridii Petruschky 1898
7. *Nocardia bovis* (Harz 1877)
Actinomyces bovis Harz 1877
Bacterium actino-cladothrix Affanassieff 1888
Actinomyces hominis (Affanassieff 1888)
Nocardia actinomyces de Toni and Trevisan 1889
Streptothrix actinomyces Rossi-Doria 1891
Oospora bovis Sauvageau and Radais 1892
Actinomyces bovis sulphureus Gasperini 1894
Cladothrix actinomyces Macé 1897
Discomyces bovis Blanchard 1900
Streptothrix hominis III Foulerton 1905 nec Foulerton 1910
Streptothrix hominis IV Foulerton 1910 nec Foulerton 1906
8. *Nocardia graminarium* (Berestneff 1897)
Streptothrix graminarium Berestneff 1891
9. *Nocardia rubra* Carabó 1894
Streptothrix rubra Kruse 1896
Nec *Actinomyces ruber* Krainsky 1914
10. *Nocardia lingualis* (Weibel 1888)
Vibrio lingualis Weibel 1888
Spirosoma lingualis Migula 1892
Streptothrix lingualis Bajardi 1900
11. *Nocardia odorifera* (Rullman 1898)
Cladothrix odorifera Rullman 1898 in sputum not in air
12. *Nocardia enteritidis* (Pottien 1902)
Streptothrix enteritidis Pottien 1902

TABLE V

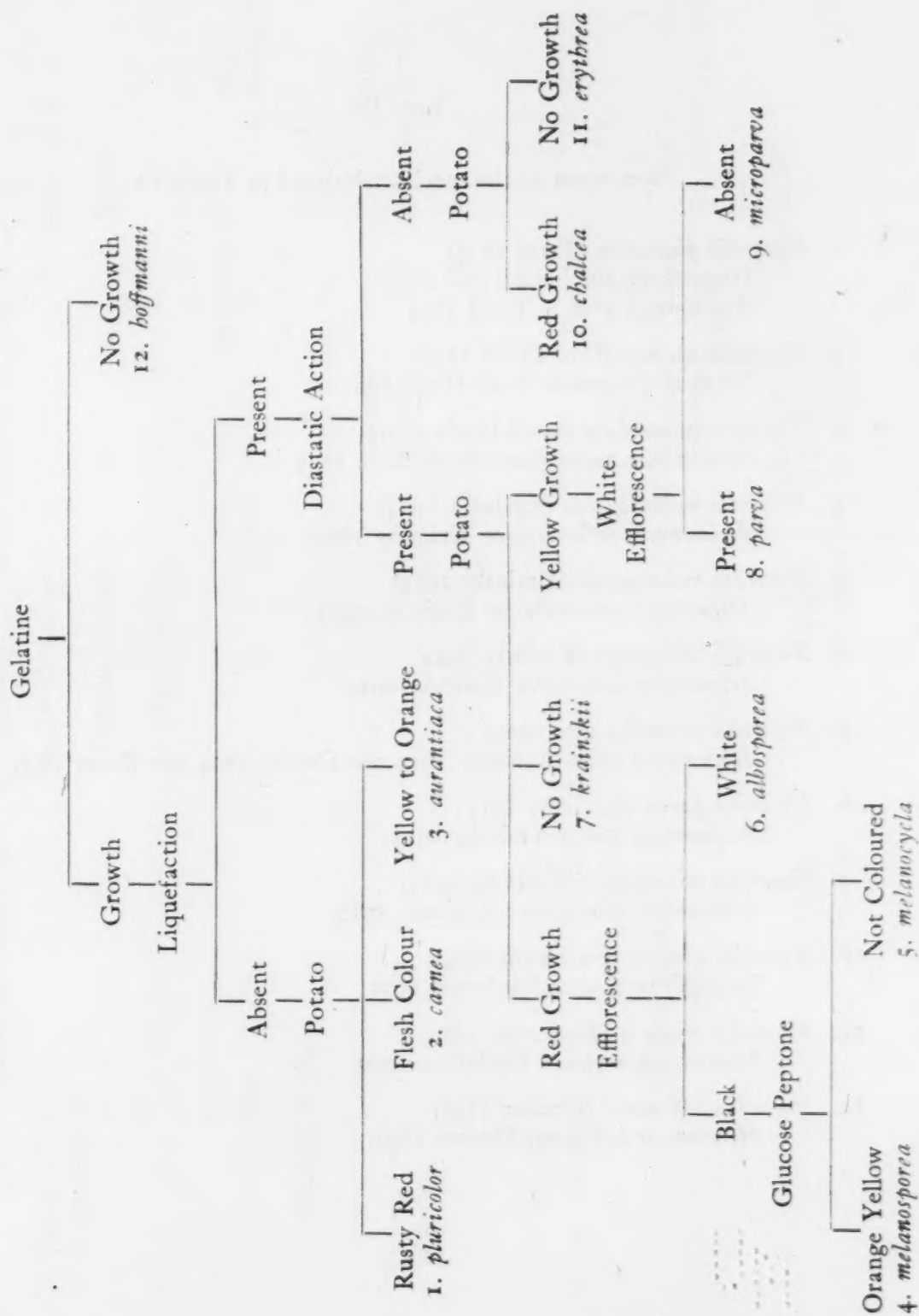
Diagnosis of the Sub-sections of the Section *Nocardia saprophytica*Sub-section 1—*Majora*

1. Grow freely under artificial conditions at 22° C., and generally at 37° C., with a few exceptions.
2. Growth usually large and spreading.
3. Development of aerial hyphae marked by a bright chalky efflorescence.
4. Earthy or mouldy smell often present in the cultures.
5. Generally peptonise gelatine and blood serum.
6. Diastatic action often present.
7. Hyphal filaments usually coarser, and branching more marked than in next series.

Sub-section 2—*Minora*

1. Grow moderately under artificial conditions at 22° C. and 37° C.
2. Growth usually moderate and circumscribed.
3. Development of aerial hyphae marked by a dull dry powdery appearance.
4. Earthy or mouldy smell either faint or absent.
5. Rarely peptonise gelatine and blood serum.
6. Diastatic action usually absent.
7. Hyphal filaments usually finer, and branching rarer than in the preceding series.

TABLE VI

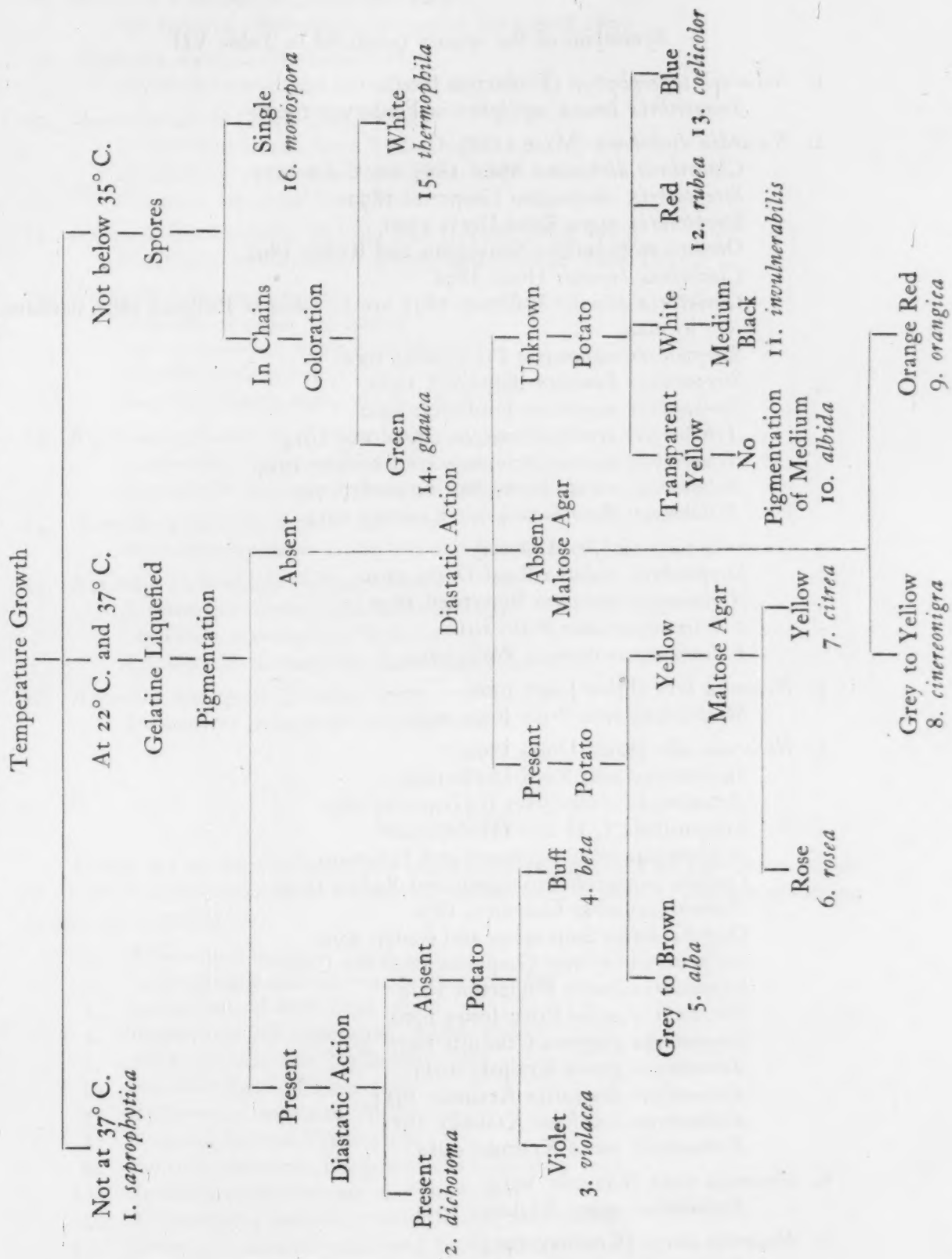
Diagnostic Table of the species of Section *Saprophytica*, Sub-section *Minora*

LIST IV

Synonyms of the species tabulated in Table VI

1. *Nocardia pluricolor* (Terni 1894)
Streptothrix pluricolor Terni 1894
Actinomyces gruberi Terni 1894
2. *Nocardia carnea* (Rossi-Doria 1891)
Streptothrix carneus Rossi-Doria 1891
3. *Nocardia aurantiaca* (Rossi-Doria 1891)
Streptothrix aurantiacus Rossi-Doria 1891
4. *Nocardia melanosporea* (Krainsky 1914)
Actinomyces melanosporea Krainsky 1914
5. *Nocardia melanocycla* (Krainsky 1914)
Actinomyces melanocyclus Krainsky 1914
6. *Nocardia albosporea* (Krainsky 1914)
Actinomyces albosporea Krainsky 1914
7. *Nocardia krainskii* new name
Actinomyces rubra Krainsky 1914 nec Carabó 1894 nec Kruse 1896
8. *Nocardia parva* (Krainsky 1914)
Actinomyces parva Krainsky 1914
9. *Nocardia microparva* (Krainsky 1914)
Actinomyces microparva Krainsky 1914
10. *Nocardia chalcea* (Foulerton 1905)
Streptothrix chalcea Foulerton 1905
11. *Nocardia erythrea* (Foulerton 1910)
Streptothrix erythrea Foulerton 1910
12. *Nocardia hoffmanni* (Gruber 1891)
Micromyces hoffmanni Gruber 1891

TABLE VII

Diagnostic Table of the species of the Section *Saprophytica*, Sub-section *Majora*

LIST V

Synonyms of the species tabulated in Table VII

1. *Nocardia saprophytica* (Foulerton 1902)
Streptothrix leucea saprophytica Foulerton 1902
2. *Nocardia dichotoma* (Macé 1888)
Cladothrix dichotoma Macé 1888 nec Cohn 1875
Streptothrix chromogena Gasperini 1890
Streptothrix nigra Rossi-Doria 1890
Oospora metschnikovi Sauvageau and Radais 1892
Cladothrix brauner Hesse 1892
Cladothrix odorifer Rullman 1895 nec *C. odorifer* Rullman 1898, parasitic in man
Streptothrix melanotica Price-Jones 1900
Streptothrix humifica Beijernick 1900
Streptothrix nigrescens Foulerton 1902
Actinomyces erythrochromogenes Krainsky 1914
Actinomyces diastaticochromogenes Krainsky 1914
Actinomyces viridochromogenes Krainsky 1914
Actinomyces flavochromogenes Krainsky 1914
3. *Nocardia violacea* (Rossi-Doria)
Streptothrix violacea Rossi-Doria 1891
Actinomyces violaceus Berestneff 1897
? *Actinomyces alni* Peklo 1910
? *Actinomyces myricae* Peklo 1910
4. *Nocardia beta* (Price-Jones 1900)
Streptothrix beta Price-Jones 1900
5. *Nocardia alba* (Rossi-Doria 1891)
Streptothrix alba Rossi-Doria 1891
Actinomyces chromogenes B Gasperini 1890
Streptothrix I, II and III Almquist
Actinomyces albus Lehmann and Neumann
Oospora guignardi Sauvageau and Radais 1892
Actinomyces albus Gasperini 1890
Oospora doriae Sauvageau and Radais 1892
Streptothrix joersteri Gasperini 1890 nec Cohn
Streptothrix leucea Foulerton 1902
Streptothrix alpha Price-Jones 1900
Streptothrix pyogenes Caminiti 1907
Actinomyces grisea Krainsky 1914
Actinomyces diastatica Krainsky 1914
Actinomyces cellulosa Krainsky 1914
Actinomyces nivea Krainsky 1914
6. *Nocardia rosea* (Krainsky 1914)
Actinomyces roseus Krainsky 1914
7. *Nocardia citrea* (Krainsky 1914)
Actinomyces griseoflavus Krainsky 1914
Actinomyces flavus Krainsky 1914
Streptothrix flava Sanfelice 1904
Streptothrix flava Brins 1899

8. *Nocardia cinereonigra* (Berestneff 1897)
 Streptothrix cinereonigra aromatica Berestneff 1897
9. *Nocardia orangica* (Berestneff 1897)
 Streptothrix orangica Berestneff 1897
10. *Nocardia albida* (Rossi-Doria 1891)
 Streptothrix albido-flava Rossi-Doria 1891
 Actinomyces farcinicus Rossi-Doria 1891
 Nocardia farcinica Rossi-Doria 1891
11. *Nocardia invulnerabilis* (Acosta and Grande Rossi 1893)
 Cladothrix invulnerabilis Acosta and Grande Rossi 1893
12. *Nocardia rubea* new name
 Actinomyces ruber (no name)
 Nec *Actinomyces ruber* Krainsky 1914
 Nec *Streptothrix rubra* Casabó 1894
 Nec *Streptothrix rubra* Kruse 1896
13. *Nocardia coelicolor* (R. Müller 1904)
 Streptothrix coelicolor R. Müller 1904
 Streptothrix coelicolor Schurman 1909
14. *Nocardia glauca* (Lehmann and Schulze)
 Actinomyces glaucus Lehmann and Schulze
15. *Nocardia thermophila* (Gilbert 1904)
 Actinomyces thermophilus Gilbert 1904
 Cladothrix thermophilis Kedzior
 Actinomyces thermophilus Berestneff 1891
16. *Nocardia monospora* (Schulze 1908)
 Actinomyces monosporus Schulze 1908

LIST VI

Incertae Sedis

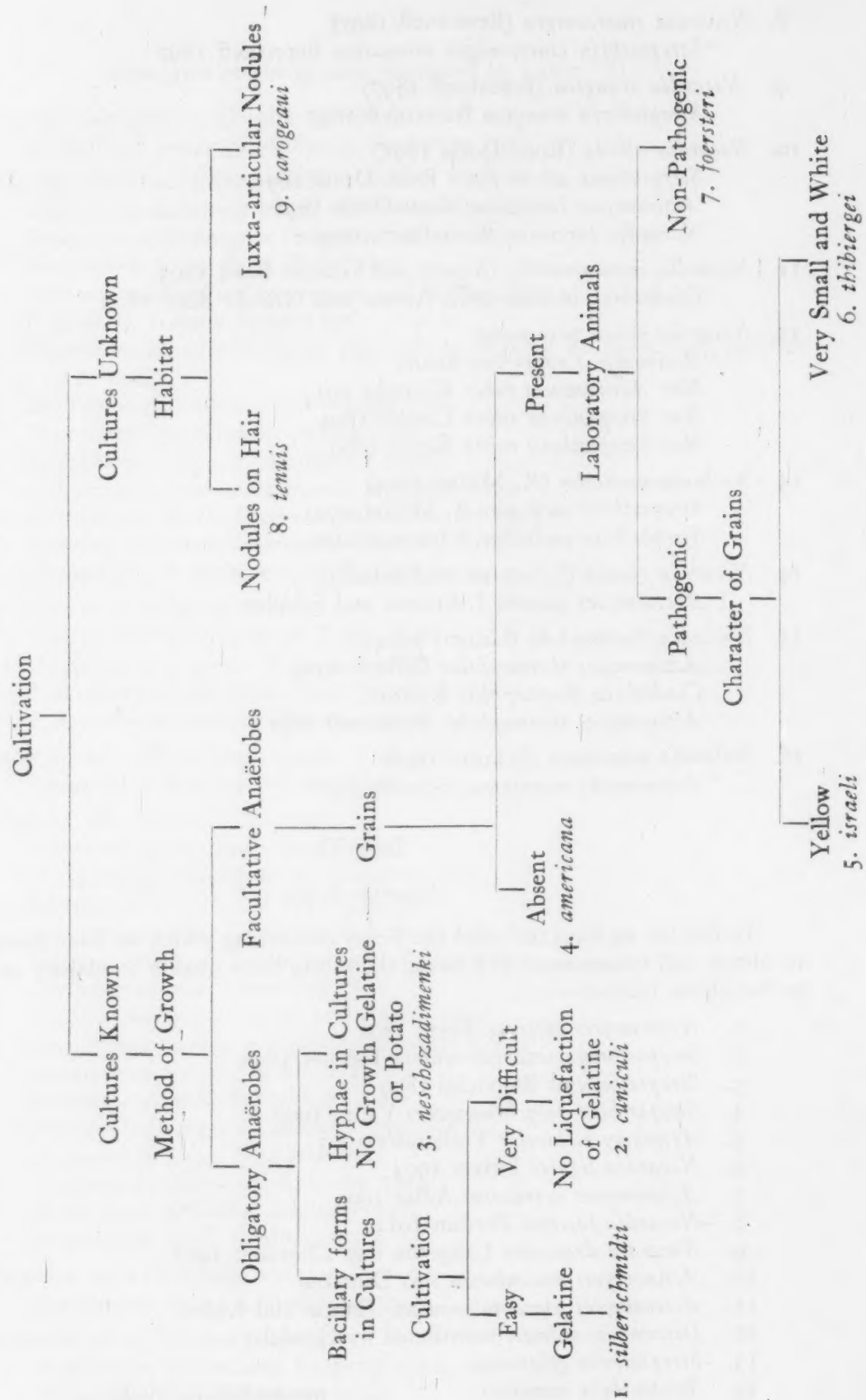
In this list we have included the forms concerning which we have been unable to obtain full information and have, therefore, been unable to classify according to the above tables :—

1. *Actinomyces lacertae* Terni 1891
2. *Streptothrix pseudotuberculosis* Flexner 1898
3. *Streptothrix* of Bonvicini 1899
4. *Streptothrix polychromogenes* Vallée 1900
5. *Actinomyces bicolor* Trollender 1903
6. *Nocardia liguire* Urizer 1904
7. *Actinomyces verrucosus* Adler 1904
8. *Nocardia lasserei* Verdun 1912
9. *Nocardia decussata* Langeron and Chevalier 1912
10. *Actinomyces musculorum suis* Duncker
11. *Actinomyces pseudotuberculosis* Hamm and Keller
12. *Discomyces holmesi* mentioned by Goedelst
13. *Streptothrix gelatinosa*
14. *Streptothrix aquatilis*
15. *Streptothrix lehmann*
16. *Streptothrix chondri* Olsen 1897

} mentioned by Peklo

TABLE VIII

Diagnostic Table of the species which may possibly belong to the genus *Gobnistreptothrix* Pinoy 1911



LIST VII

Synonyms of the species tabulated in Table VIII

1. *Cohnistreptothrix silberschmidti* new name
This name is given to distinguish the obligatory anaërobic *Streptothrix* found by Silberschmidt in 1900, in Dacryocystitis, and described in the *Centralblatt für Bakteriologie*, XXVII, and further cases in *Zeitschrift für Hygiene* (1901), XXXVII.
2. *Cohnistreptothrix cuniculi* (Schmorl 1891)
Streptothrix cuniculi Schmorl 1891
Actinomyces cuniculi Gasperini 1894
Streptothrix necrophora Kitt 1906
? *Bacillus necroseos* Salmonsens
? *Necrosis bacillus* of Bang
? *Bacillus diphtherae vitulorum* Flügge
? *Bacillus necrophorus* Flügge
3. *Cohnistreptothrix neschadimenki* new name
This name is given to distinguish the obligatory anaërobic *Streptothrix* found by Neschadimenko, in 1908, in human pus, and described in the *Centralblatt für Bakteriologie*, XLVI.
? *Coccobacillus pseudo-actinomycosis polymorphus* Berestneff 1898
4. *Cohnistreptothrix americana* new name
This name is given to distinguish the *Streptothrix* which only grows under partial anaërobic and aërobic conditions, and obtained from a liver abscess by Bloomfield and Bayne-Jones in 1915, and described in *Johns Hopkins Hospital Bulletin*, XXVI, No. 292.
5. *Cohnistreptothrix israeli* (Kruse 1896)
Streptothrix israeli Kruse 1896
Streptothrix spitzi Lignières 1903
Possibly the *Streptothrices* described by Doyen in 1891, by Jurinka in 1896, and some of those by Silberschmidt in 1901, by Schukewitsch in 1902, by Doepke in 1903, and by Wright in 1904.
6. *Cohnistreptothrix thibiergei* (Ravaut and Pinoy 1909)
Discomyces thibiergei Ravaut and Pinoy 1909
7. *Cohnistreptothrix foersteri* (Cohn 1874)
Streptothrix foersteri Cohn 1874
Leptothrix oculorum Sorokin 1881
Oospora foersteri Sauvageau and Radais 1892
Streptothrix aureus Du Bois de Saint Séverin 1895
Streptothrix foersteri Kruse 1896
The aërobic *Streptothrix* of Silberschmidt obtained from a case of Dacryocystitis 1901.
? *Streptothrix radiatus* Namyslowski 1909
? *Streptothrix cerebriiformis* Namyslowski 1909
8. *Cohnistreptothrix tenuis* (Castellani 1911)
Nocardia tenuis Castellani 1911
9. *Cohnistreptothrix carogeaui* (Cougerot 1909)
Discomyces carogeaui Cougerot 1909
Nocardia carogeaui Castellani and Chalmers 1913

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EXPLANATION OF PLATES

Most of these illustrations may, with advantage, be examined by means of a reading lens.

PLATE VIII

- Fig. 1. Sudanese Actinomycotic Mycetoma. $\times 1.5$ diameters. Photograph.
- Fig. 2. Eosinophile bodies, often called Fuchsin—or Botryomycotic bodies. $\times 1,440$ diameters. Photomicrograph.
- Fig. 3. A grain removed from the Mycetoma depicted in fig. 1. $\times 20$ diameters. Photograph.
- Fig. 4. Aërobic growth for five days at 30° C. on Sabouraud's preservative medium, of the fungus *Nocardia convoluta*, derived from grains taken from the Mycetoma depicted in fig. 1. Photograph.
- Fig. 5. *N. convoluta*. Growth on clear maltose agar, watch-glass method, for eight days at 37° C. Photograph.
- Fig. 6. Fresh preparation in normal saline from the grain depicted in fig. 3. Showing nocardial hyphae. Photomicrograph.
- Fig. 7. *N. convoluta*. Growth on potato for four days at 37° C. Photograph.
- Fig. 8. *N. convoluta*. Aërobic growth on Sabouraud's preservative medium for eight days at 30° C. Photograph.
- Fig. 9. *N. convoluta*. Anaërobic growth on Sabouraud's preservative medium for twelve days at 30° C. Photograph.
- Fig. 10. *N. convoluta*. Growth on inspissated ox-blood serum for seven days at 30° C. Photograph.
- Fig. 11. *N. convoluta*. Growth on inspissated ox-blood serum for twelve days at 30° C., showing commencing liquefaction of the medium. Photograph.



Fig. 1

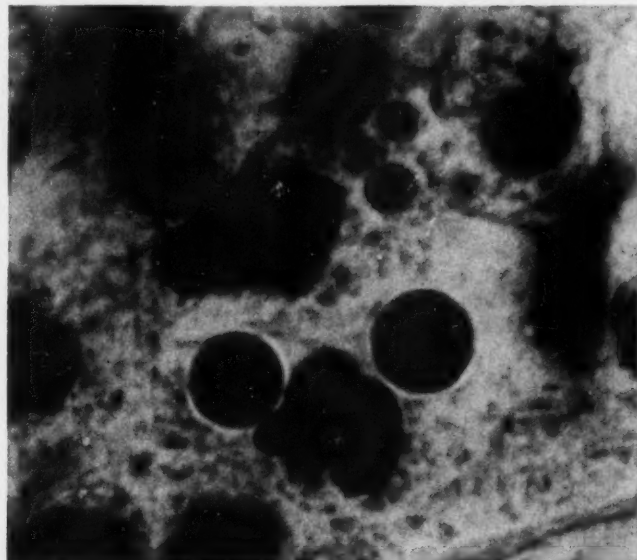


Fig. 2

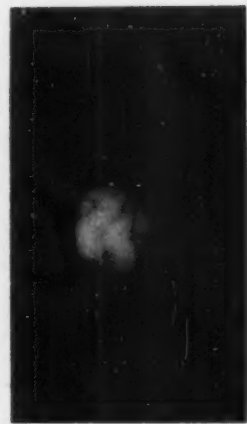


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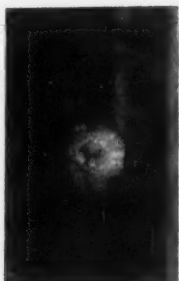


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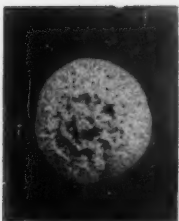


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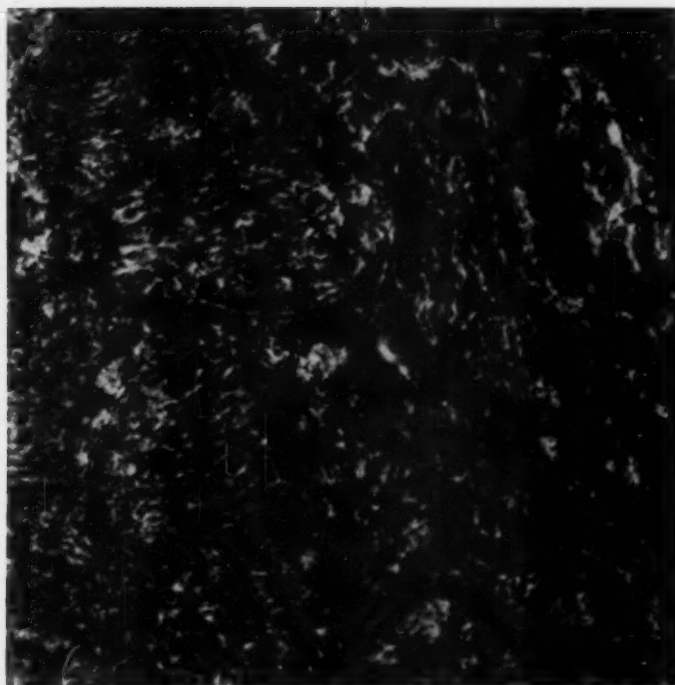


Fig. 6



Fig. 7



Fig. 8



Fig. 9



Fig. 10



Fig. 11

PLATE IX

- Fig. 12. General view of an old fungal mass. $\times 30$ diameters. Photomicrograph.
- Fig. 13. Vessels, cell masses and fat cells in matrix. $\times 90$ diameters. Photomicrograph.
- Fig. 14. Vessel showing peri- and endarteritis. $\times 400$ diameters. Photomicrograph.
- Fig. 15. Cells accumulated near a vessel, as depicted in fig. 13. $\times 500$ diameters. Photomicrograph.
- Fig. 16. Fungus escaping from giant cell. $\times 400$ diameters. Photomicrograph.
- Fig. 17. Young fungal mass. Note the remains of the giant cell and also the polymorphonuclear cells of the fibro-cellular sheath. $\times 400$ diameters. Photomicrograph.

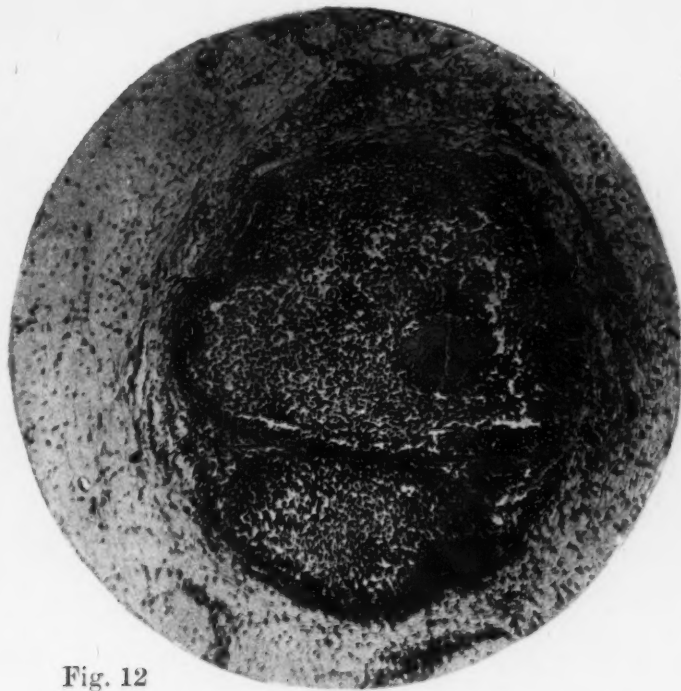


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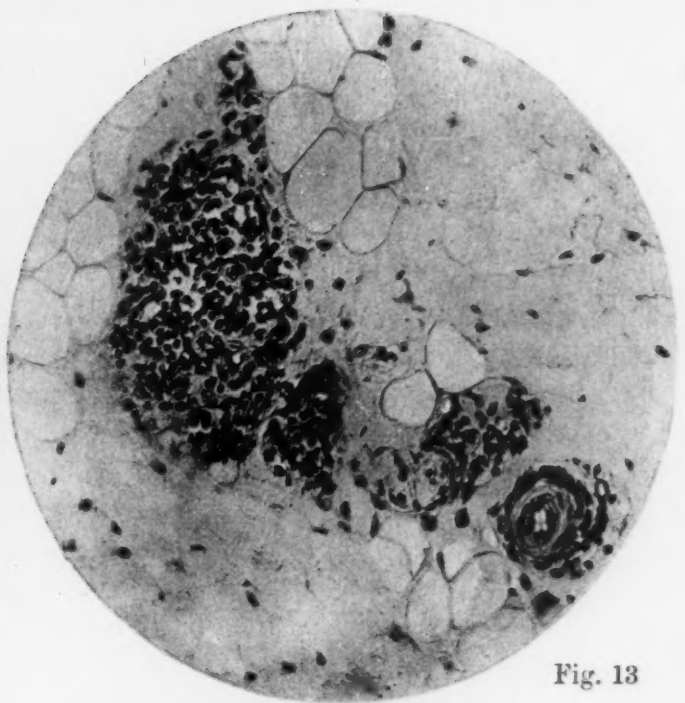


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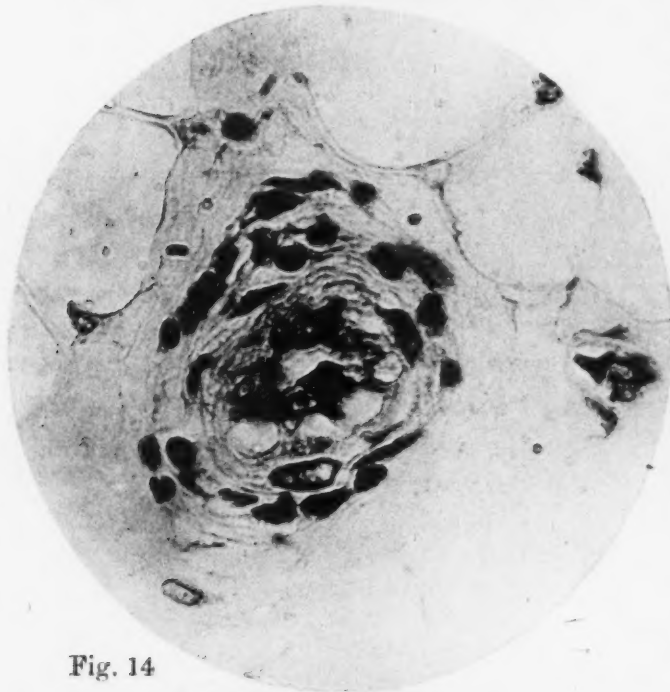


Fig. 14

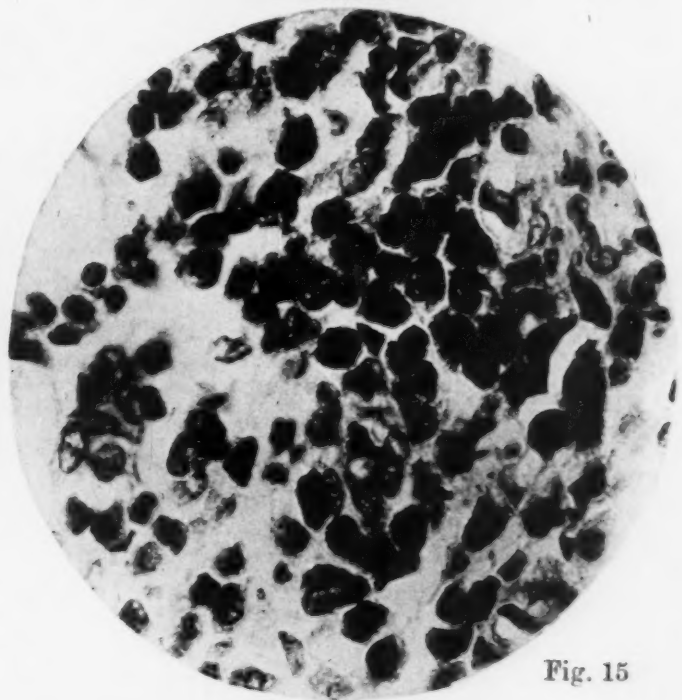


Fig. 15

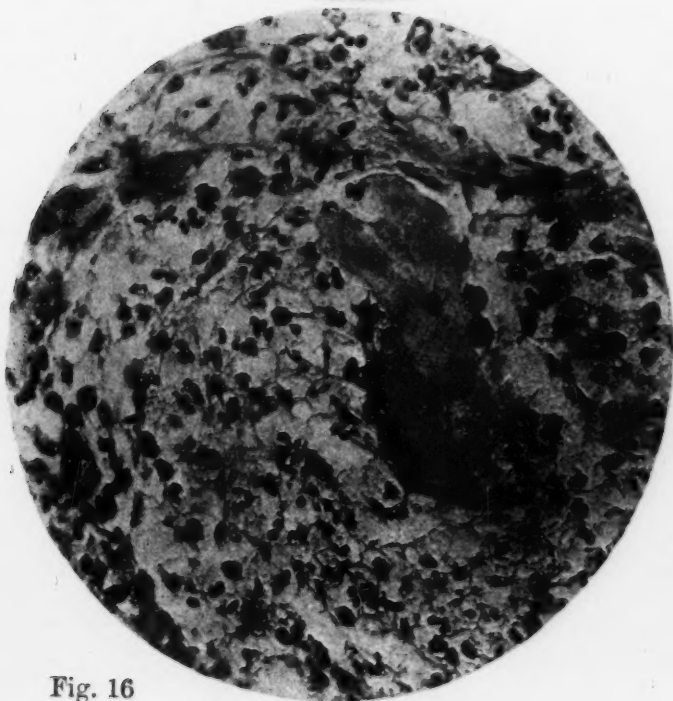


Fig. 16

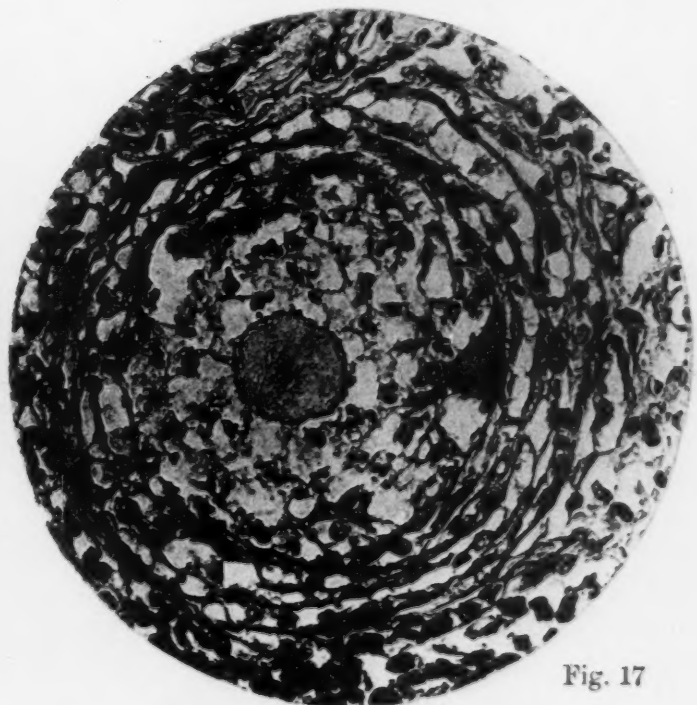


Fig. 17

PLATE X

Fig. 18. Higher power view of fig. 12. $\times 82$ diameters. Photomicrograph.

Fig. 19. Giant cells containing portion of the fungus. $\times 940$ diameters. Photomicrograph.

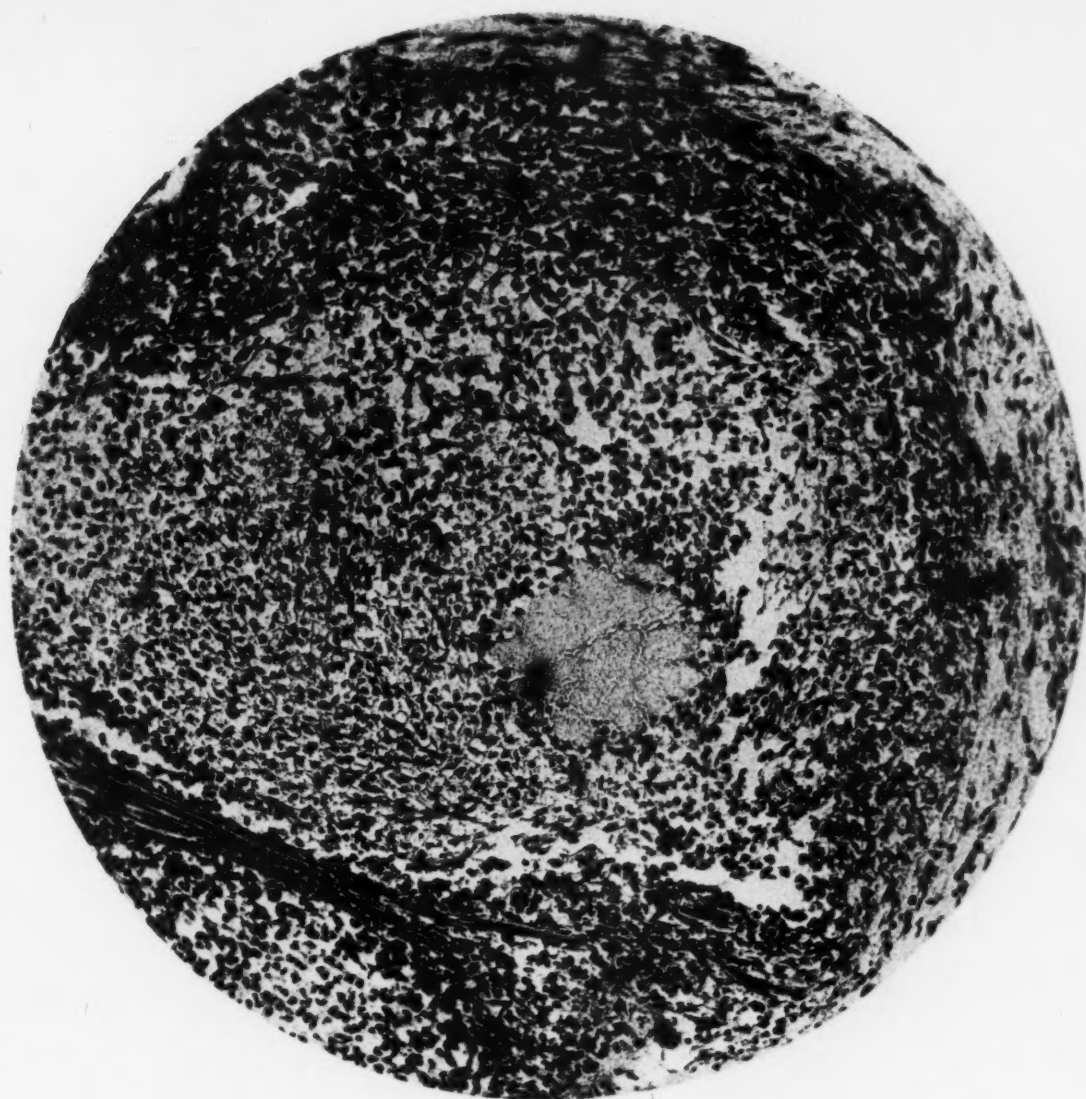


Fig. 18

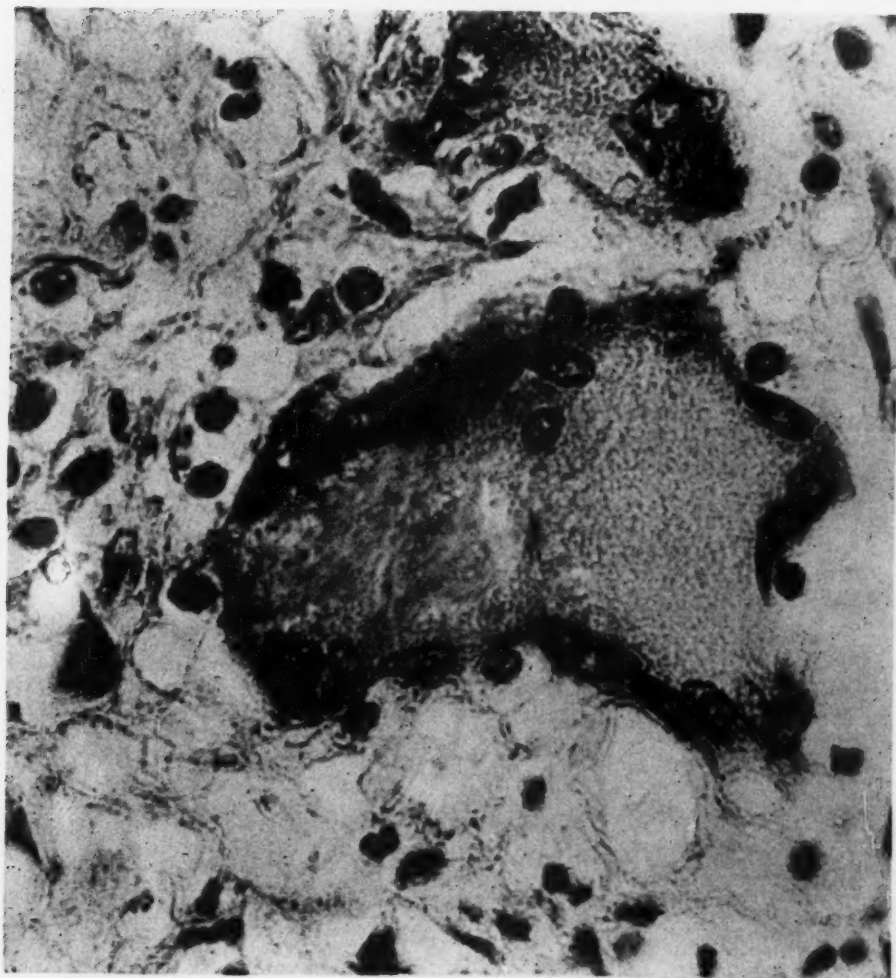


Fig. 19

PLATE XI

- Fig. 20. *N. convoluta*. Hypha showing beading, also commencing separation into three portions. Blood serum culture at 30° C. × 1,500 diameters. Photomicrograph.
- Fig. 21. *N. convoluta*. Hypha showing branching. Note also forms with commencing arthrospores, also bacillary and coccal forms. Blood serum culture at 30° C. × 1,500 diameters. Photomicrograph.
- Fig. 22. *N. convoluta*. Young hypha showing cytoplasm and beading. Blood serum culture at 30° C. × 2,800 diameters. Photomicrograph.
- Fig. 23. *N. convoluta*. Growth on Sabouraud's maltose agar for five days at 37° C. × 15 diameters. Photograph.
- Fig. 24. *N. convoluta*. Hypha with Gram-positive and Gram-negative lengths; the former filled with cytoplasm, which is lacking in the latter. Blood serum culture. × 1,000 diameters. Photomicrograph.
- Fig. 25. *N. convoluta*. Showing breaking up and absorption of the Gram-positive material of the hyphae prior to the stage depicted in fig. 24, which leads to the fragmentation of the hyphae. × 1,000 diameters. Photomicrograph.
- Fig. 26. *N. convoluta*. Hyphae with chains of spores.
- Fig. 27. *N. convoluta*. Arthrospores.
- Fig. 28. *N. convoluta*. Young growth on agar-agar at 30° C. for forty-eight hours. × 4 diameters. Photograph.
- Fig. 29. *N. convoluta*. Growth on glucose agar at 30° C. for three days. × 2 diameters. Photograph.



Fig. 20

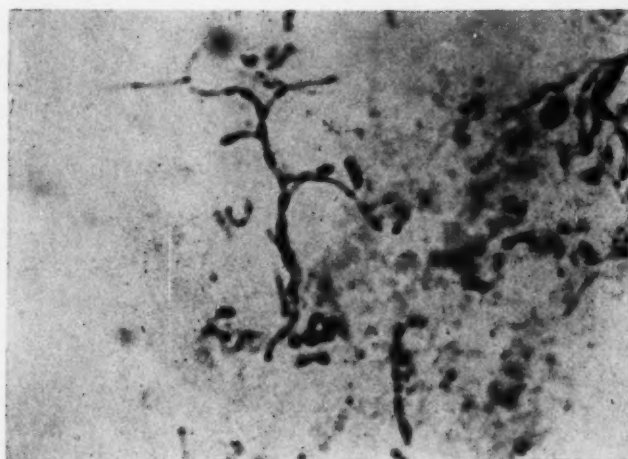


Fig. 21

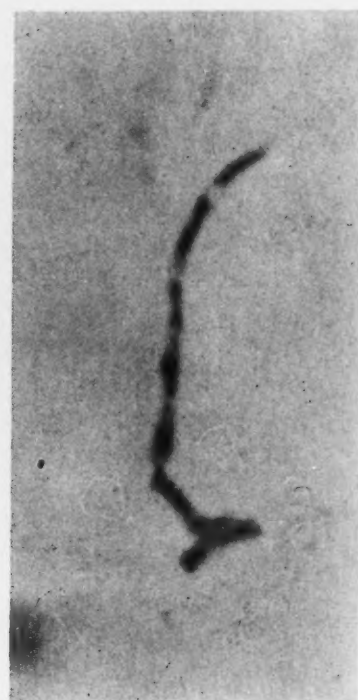


Fig. 22



Fig. 23

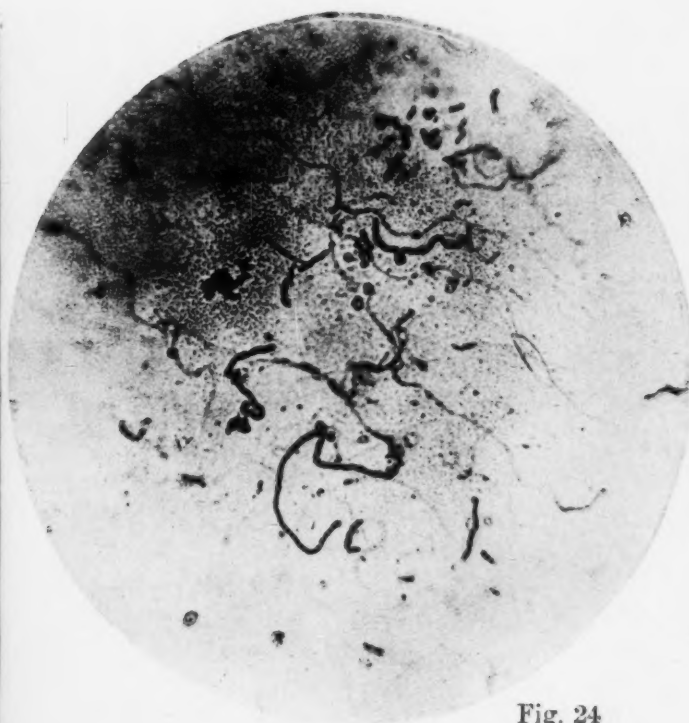


Fig. 24



Fig. 25

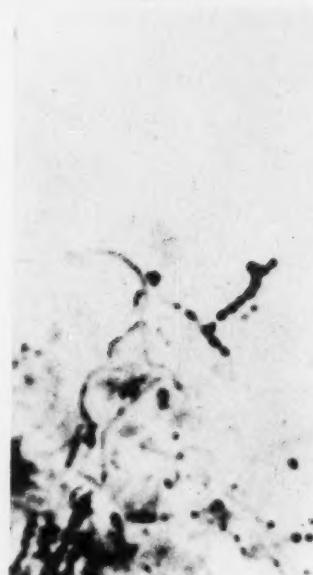


Fig. 26



Fig. 27

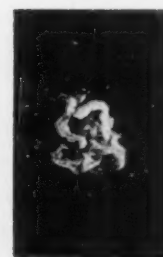


Fig. 28

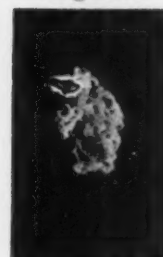
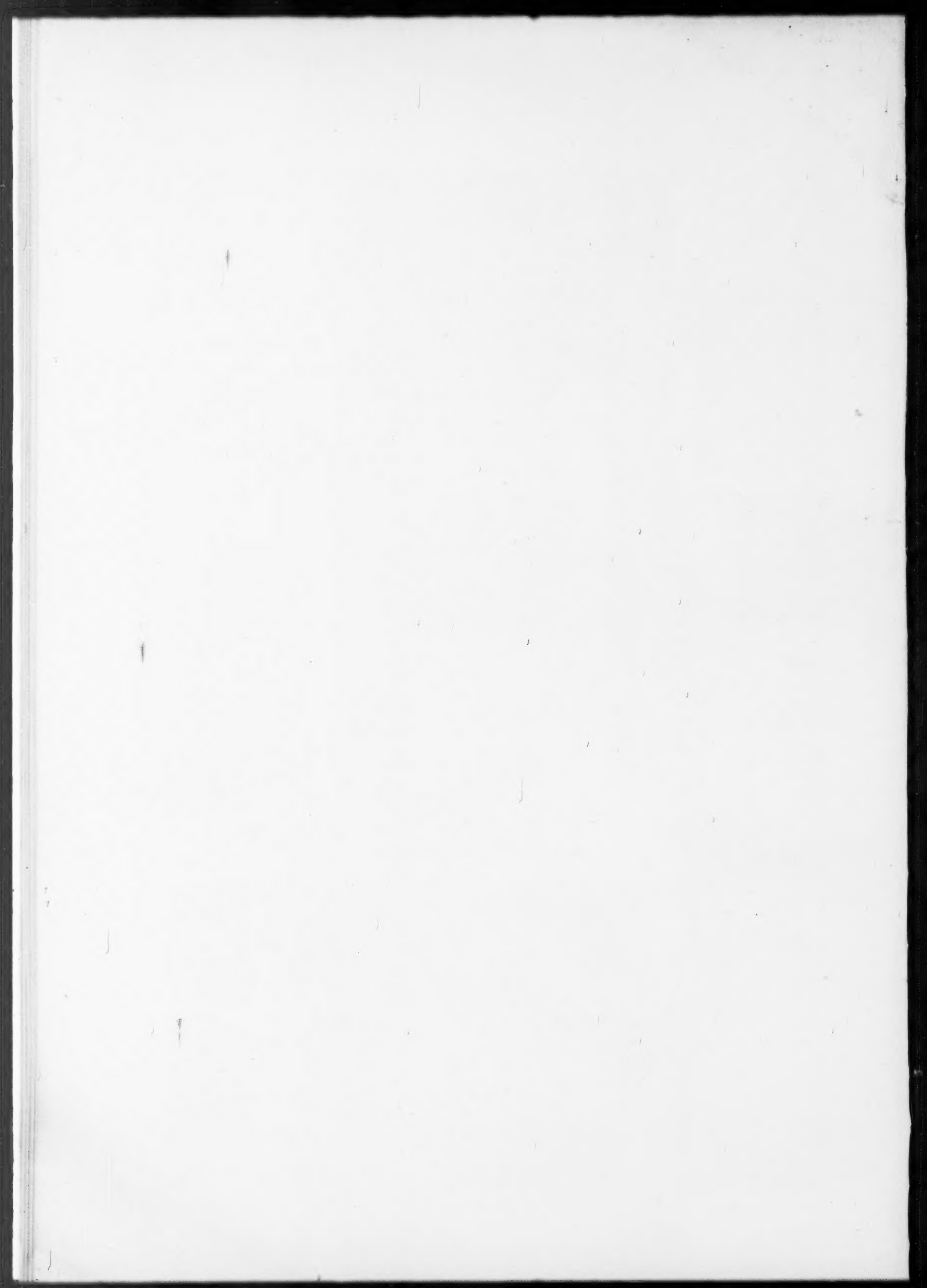


Fig. 29



A FOURTH VARIETY OF TRICHONOCARDIASIS, WITH A NOTE ON THE CULTIVATION OF *NOCARDIA TENUIS*
(CASTELLANI, 1911)

BY

J. W. SCOTT MACFIE, D.Sc., M.B.

WEST AFRICAN MEDICAL STAFF

(Received for publication 15 August, 1916)

INTRODUCTION

Trichonocardiasis is by no means uncommon in West Africa, but, so far as I am aware, its occurrence in this part of Africa has not previously been recorded, with the exception of a brief reference to Trichonocardiasis flava in Europeans on the Gold Coast contained in the excellent account of the occurrence of this condition in the Sudan published by Chalmers and O'Farrell (1913). In its introduction this paper was claimed to be the first record of the occurrence of Trichonocardiasis in Africa, but further on (p. 527) mention was made of 'a condition resembling Trichonocardiasis flava in Europeans on the Gold Coast, West Africa,' observed by one of the authors as long ago as 1898. As Castellani did not describe the disease until 1911 the diagnosis of the condition observed in 1898 was presumably retrospective, and it is perhaps of some interest to confirm the record.

This disease, which affects the shafts of the hairs in the axilla and groin, is caused by a fungus *Nocardia tenuis*, either alone or associated with a chromogenic organism. Three varieties are recognised, namely, Trichonocardiasis flava in which only the fungus is present, Trichonocardiasis nigra in which it is associated with *Micrococcus nigrescens*, and Trichonocardiasis rubra in which it is associated with *Micrococcus castellanii*. A fourth variety, which from its brownish colour might appropriately be called Trichonocardiasis fusca, occurs on the Gold Coast, and a short description of this form is given in this paper.

TRICHONOCARDIASIS FUSCA

In a case of Trichonocardiasis examined recently the nodules on the hairs were of a brownish or light brick-red colour, quite distinct from the yellow tint of Trichonocardiasis flava or the rich red colour of Trichonocardiasis rubra.

Dr. A. Ingram, who very kindly sent me the materials from this case and to whom I have much pleasure in acknowledging my thanks, stated that the patient was a European who had been aware of the presence of the affection for the last three years. When the patient was in England the nodules disappeared, but always reappeared a month or two after his return to West Africa. The hairs affected were those of the axillae.

Scrapings from the hairs, when sown on ordinary agar or ascitic agar, produced an abundant crop of yellow colonies. The organism itself was colourless, non-motile, and had the characteristic appearance of a diplococcus. It stained well with all the ordinary reagents, but when treated by Gram's method the result was not uniform: some of the cocci showed a positive reaction, others a negative, and not infrequently one segment of a diplococcus was seen to have retained the stain while the other had been completely decolourised. The same peculiarity was observed by Chalmers and O'Farrell in the case of *Micrococcus castellanii*.

On agar, glucose agar, and ascitic agar the diplococcus grew well, producing yellow colonies of a shade similar to that depicted by Chalmers and O'Farrell as typical of young cultures of *M. castellanii*, and which they describe as being lemon-chrome coloured. In this case, however, the colonies never developed a red colour, no matter how old they were nor on what medium they were growing; indeed, in very old cultures the yellow colour became if anything slightly paler. In nutrient broth an abundant growth took place, producing a general turbidity and throwing down a white precipitate, but the yellow colour was not obvious in this medium. No indol was produced in peptone water, and no fluorescence in neutral red medium. Neither acid nor gas was produced after a week's cultivation in the following sugary and alcoholic peptone media: glucose, laevulose, galactose, maltose, lactose, saccharose, dulcitate, and mannite. Unfortunately it was

impossible to test these reactions further owing to a lack of the rarer carbohydrates, &c., which are at present unobtainable.

So far as it was possible to test its properties, this organism seemed to resemble in its general characters the cocci associated with the red and black varieties of trichonocardiasis, but differed from them in the colour of the pigment it produced.

OTHER FORMS OF TRICHONOCARDIASIS

Europeans on the Gold Coast are not infrequently affected by trichonocardiasis, but as the disease causes little or no inconvenience, and as it usually attacks the hairs of the axillae which are not always easy to scrutinize, it is often overlooked by the patient himself. It is probable, therefore, that the condition is much more common than is generally supposed.

Up to the present I have observed, in addition to the variety described above, the two forms known as *Trichonocardiasis flava* and *Trichonocardiasis rubra*. From the latter variety a diplococcus has been isolated which conforms morphologically and culturally to the description of *Micrococcus castellanii* given by Chalmers and O'Farrell. I have not yet seen a case of the black variety, *Trichonocardiasis nigra*.

With regard to the natives, it is difficult to determine to what extent they are subject to the disease since most of the races shave the axillae. Chalmers and O'Farrell suggest that this practice may have originated, for one reason, because the occurrence of trichonocardiasis had been observed by the native doctors. If this was so, the fact seems to have been forgotten, for now-a-days a native, if asked why he shaves the axillae, will generally reply that it is because it prevents excessive perspiration.

The pathology and microscopical anatomy of this affection have been fully described by Chalmers and O'Farrell. Only the shafts of the hairs appear to be affected, and the nodules when discovered by accident, as they generally are, are often mistaken for dirt or dried sweat until it is found that no amount of washing will remove them. It has been stated that the hairs are not affected by the growth, but of this I am uncertain, as I have observed that the diseased hairs

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in Europeans appeared to be discoloured or greyer than the healthy ones, and Dr. Ingram, in forwarding to me the materials from the case of *Trichonocardiasis fusca*, stated that in this patient the hairs in the right axilla, which were heavily infected, were nearly all grey, whereas those in the left axilla, which were but little affected, retained their pigment.

The infection, as is well known, dies out in a temperate climate, but it is liable to reappear on returning to the tropics. If it really were only the shafts of the hairs that were affected, it might be supposed that a rapid and efficient means of dealing with the condition would be shaving. This has been tried in some cases, but unless other means were adopted at the same time, the disease reappeared as soon as the hairs had re-grown to a length of half or three-quarters of an inch.

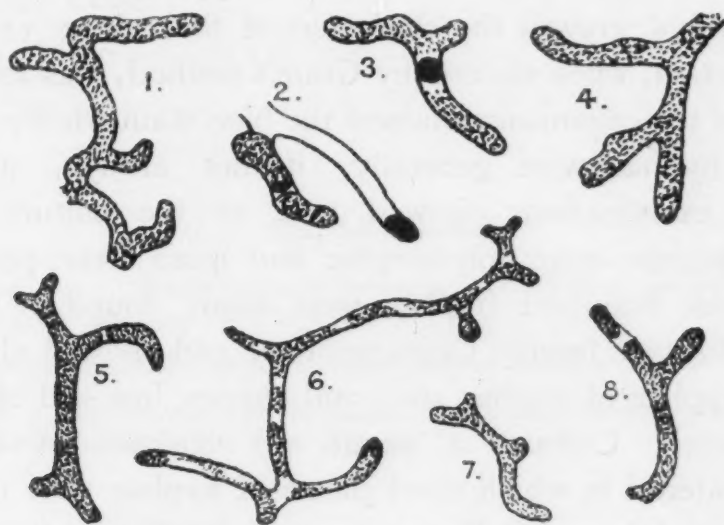
THE CAUSAL ORGANISM—*NOCARDIA TENUIS*

Scrapings from the infected hairs of all the cases I have examined have shown great numbers of hyphae, the majority of which were similar to those described by Chalmers and O'Farrell, namely, 'narrow, elongated, unbranched, non-septate rods resembling bacilli.' The hyphae usually stained deeply, but some only faintly, as though they were mere empty shells. Intensely stained granules were common, and occurred both at the ends and in the middle of the hyphae. The organism was Gram-positive and not acid-fast, and appeared to be identical with *Nocardia tenuis*, the causal agent of *Trichonocardiasis* described by Castellani (1911).

Chalmers and O'Farrell state that they did not meet with any branched forms in the nodules on the hairs, but they observed them in the slight growth they obtained in hanging drop cultures of blood serum and saline solution. Castellani and Chalmers (1913), however, state that the hyphae found in the nodules are 'occasionally branching.' In the cases I have examined in the Gold Coast, branched hyphae were always easily found (see figs. 1 to 4).

Measurements of such an organism are necessarily somewhat unsatisfactory owing to the diversity of forms found in the scrapings from the hairs. The lengths, 2μ to over 7μ , given by Chalmers and O'Farrell, and 4μ to 10μ given by Castellani and Chalmers, were

of course greatly exceeded in my cases in those hyphae which were branched. There seems to be some uncertainty about the breadth of the hyphae of *Nocardia tenuis*. According to Castellani and Chalmers (1913) 'they are rather thin, 1 to $1\frac{1}{2}\mu$,' but according to Chalmers and O'Farrell (1913) the average breadth is 'from 0.14μ to 0.3μ .' The latter authors, however, figure (Plate XXXVI, figs. 10 and 12; Plate XXXVII, fig. 21) some hyphae which appear to measure 0.6μ . The breadth of the hyphae in my cases was about 0.6μ .



FIGS. 1 to 4.—Branched hyphae in scrapings from axillary hairs of a case of *Trichonocardiasis flava*. $\times 3000$.

FIGS. 5 to 8.—Branched hyphae from a culture a month old from the same hairs. $\times 3000$.

Nocardia tenuis has not previously been cultivated, but as has already been mentioned, Chalmers and O'Farrell observed some growth in hanging drops of equal parts of human blood serum and 0.85 per cent. normal saline solution.

Scrapings from the infected hairs of one of my cases of *Trichonocardiasis flava* were soaked in absolute alcohol and then planted on ascitic agar. After a few days' incubation at 37°C . a slight translucent growth was observed in one tube which on examination proved to be a pure culture of long, narrow hyphae. The colony grew slowly, and remained translucent and almost invisible. Sub-cultures on ascitic agar showed some growth within twenty-four hours, but the colonies were never luxuriant and spread

slowly over the surface of the medium as a thin transparent growth, the centre only having a slightly opaque ground-glass appearance. No difficulty was experienced in keeping the strain going on this medium, but sub-cultures on to ordinary agar were invariably unsuccessful.

The growth on ascitic agar at first consisted of bacillary bodies and long slender hyphae about 0.6μ in diameter. They were non-motile Gram-negative, and not acid-fast. The hyphae were unbranched, not septate, often of great length, and at the ends of some of the filaments a few short pieces appeared to be separated. After two days' growth the characters of the culture were similar, but the reaction, when stained by Gram's method, was less uniform, and some of the organisms retained the blue stain wholly or in part. The long hyphae were generally, if not always, unbranched. Successive examinations showed that as the culture aged the organism became more polymorphic and more Gram-positive, and after a week branched hyphae were easily found. Cultures a fortnight old were largely Gram-positive, and showed clubbed and branched hyphae of various sizes and shapes, but still of about the same diameter. Cultures a month old consisted of a mass of granular material in which short pieces of hyphae were distributed. These fragments were of all sizes, coccoid, bacillary, often branched, and very similar to the bodies found in the scrapings from infected hairs. They were Gram-positive, about 0.6μ broad, and the larger branched pieces showed the hypha in process of breaking up (see figs. 6 and 8). So closely did the organism in such a culture resemble that seen in smears made from scrapings of the hairs of the original case, that it could hardly be doubted but that they were the same.

On examining infected hairs stained by Gram's method a notable difference in reaction was observed. The larger nodules and those at the bases of the more heavily infected hairs retained the stain so that it was usually impossible to make out any details, but in the least dense portions Gram-positive organisms could sometimes be distinguished. The very young nodules and the areas of the hairs in which the infection was just commencing were, on the contrary, either completely or partially decolourised, and in specimens that had been counter-stained Gram-negative filaments

and bacillary bodies were seen in abundance. In a single hair, starting from its tip, all the stages observed in cultures on ascitic agar could be traced, from long Gram-negative filaments to short Gram-positive bodies of the type named by Castellani *Nocardia tenuis*. It must be concluded, therefore, that the characters of *Nocardia tenuis* as seen in the nodules on the hairs of a well-established case of trichonocardiasis represent only one phase in the life of this fungus, the nodules in fact corresponding to a culture of some considerable age.

ACCRA, GOLD COAST,
July, 1916.

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The first part of the paper is devoted to a discussion of the general principles of the theory of the structure of the atom. It is shown that the structure of the atom is determined by the laws of quantum mechanics, and that the laws of quantum mechanics are based on the principle of the conservation of energy.

In the second part of the paper, the author discusses the application of the theory of the structure of the atom to the study of the properties of matter. It is shown that the theory of the structure of the atom can be used to explain the properties of matter, such as the properties of the elements and the properties of the compounds.

The third part of the paper is devoted to a discussion of the experimental methods used to study the structure of the atom. It is shown that the experimental methods used to study the structure of the atom are based on the principles of quantum mechanics, and that the experimental methods used to study the structure of the atom are based on the principle of the conservation of energy.

In the fourth part of the paper, the author discusses the results of the experiments and the conclusions drawn from them. It is shown that the results of the experiments are in good agreement with the predictions of the theory of the structure of the atom, and that the conclusions drawn from the experiments are in good agreement with the predictions of the theory of the structure of the atom.

OBSERVATIONS ON URINARY AMOEBIASIS

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INTRODUCTION

As a remote complication of amoebic dysentery there is no reason why the genito-urinary tract should not be affected, since it is recognised that the protozoal parasite from the large intestine may find its way through the portal system, to the liver most commonly of course, but also, as Rogers (1913) has pointed out, 'to the spleen or even more distant parts, such as the brain and other tissues, in any of which it may set up inflammatory processes ending in suppuration.' There is also a more direct route, to which Craig (1911) has called attention in his statement that there is 'no reason why any of the species [of *Entamoeba*] occurring in the intestine should not occasionally be found in the urine, reaching it through fistulae between the bladder and intestine, which may occur in cases of amoebic dysentery, or intestinal amoebae might reach the kidney or bladder through the surrounding tissues or through the blood stream.' Considering the wide distribution and frequency of occurrence of amoebic dysentery, it is a somewhat remarkable fact that very few cases have been recorded in which amoebae were found in the urine.

PREVIOUS RECORDS

The earliest record of urinary amoebiasis appears to be that of Baelz (1883), who described an amoeba found in the blood-stained urine and in the vagina of a patient in Japan. This organism, called *Amoeba urogenitalis*, measured from 23μ to 50μ in diameter, was actively motile, and extruded pseudopodia that were short and blunt. Its cytoplasm was granular in appearance, it was phagocytic for red blood corpuscles, and it possessed a vesicular nucleus.

Similar cases appear to have been recorded by Jürgens, Kartulis, Posner, Wijnhoff, and Jeffries, but in the literature at our disposal here at Accra we are not able to consult the original papers. Craig (1911) in referring to the cases described by Baelz, Jürgens, Posner, and Kartulis, considers that it is still undecided whether the organism (*Entamoeba urogenitalis*) 'is entitled to specific rank, most authorities believing that the amoebae described by these authors were either *Entamoeba histolytica* or *Entamoeba tetragena*.' Fantham (1916), in a short memorandum in the 'British Medical Journal,' gives the following particulars of these cases:—'Jürgens found small mucous cysts, containing amoeboid bodies, in the bladder of an old woman suffering from chronic cystitis; they were also found in the vagina. Kartulis (1893) observed similar organisms in the sanguineous urine of a woman* suffering from a tumour of the bladder; the organisms measured 12μ to 20μ , and exhibited slow pseudopodial movements, and a nucleus and vacuoles were seen after staining. Posner's case, a man, also passed blood stained urine, in which amoeboid granular bodies, about 50μ by 28μ , were present. The amoebae exhibited change of shape, and contained one or more nuclei as well as red blood corpuscles. The patient was under observation for over a year, during which the attacks recurred, and Posner concluded that the amoebae had penetrated into the pelvis of the kidney. Wijnhoff observed four cases of amoeburia in Utrecht, and Jeffries (1904) found similar cases in the United States.'

Craig (1911) mentions a case of infection of the bladder with *Entamoeba histolytica*, in which at the autopsy he found a minute fistula between the ulcerated intestine and the bladder.

Fischer (1914) found amoebae indistinguishable from *E. tetragena* in the urine of a Chinaman at Shanghai. The patient complained of painful micturition, and passed yellow and very acid urine containing a thick white sediment in which numerous amoebae were found. The parasites were from 15μ to 25μ in diameter; but no cysts were seen. There was no history of dysentery. Unfortunately the patient refused to remain under observation, so that a complete study of the case could not be made.

Lynn (1914) examined a somewhat similar case in a native man in Costa Rica. The patient complained of incontinence of urine and

* Man in the original.—EDD.

a burning sensation on micturating. There was a history of gonorrhoea two years previously, and a prostatic stricture was found. The urine contained blood, pus, and motile amoebae. No details were recorded of the morphology of the amoebae, but they were stated to have been *Entamoeba tetragena*. The infection was thought to have been conveyed by means of a syringe with which the patient washed out his own bladder having previously been used for rectal lavage. No amoebae were, however, found in the stool, although pus and blood were present, but as two doses of emetine had already been administered before this examination was made, it cannot be regarded as conclusive. There was no connexion between the intestinal tract and the bladder. The patient responded well to treatment (presumably emetine), and made a rapid recovery.

Finally, quite recently, Ward, Coles, and Friel (1916) have examined at Bournemouth and have recorded briefly a case of jaundice with albuminuria from Mudros, in which amoebae were found in the urine. The amoebae were 'light yellow-greenish bodies, circular or pear-shaped in outline, and of a markedly granular appearance.' There was no appreciable differentiation of endoplasm and ectoplasm, but some specimens that were watched carefully were seen to protrude a relatively clear pseudopodium that contrasted sharply with the semi-opaque granular endoplasm. The nucleus was easily seen, and was usually relatively large. The amoebae measured from 8μ or less to 33μ or more in diameter, but probably averaged about 20μ to 25μ . No cysts were seen in the urine. Cysts of *Entamoeba coli* were present in the faeces.

The authors consider this organism to be 'totally unlike *Entamoeba coli* or *Entamoeba histolytica*,' and proposed for it the new name *Amoeba urinae granulata*, but the details at present available are insufficient to distinguish the parasite from those previously described as occurring in the urine, and we agree with Fantham (1916) that the provisional name 'seems hardly necessary.'

The references enumerated above do not, of course, pretend to be a complete bibliography, but they are sufficient to show that amoebae have been detected in the urine in a number of cases, in white and yellow and dark skinned races, and in widely separated parts of the world, from China and Japan to Bournemouth and Utrecht.

CASES RECENTLY EXAMINED ON THE GOLD COAST

During the last two years we have examined three cases of genito-urinary amoebiasis at Accra, Gold Coast Colony, West Africa.* Two of the patients were negroes and one a European; all three were males.

The two cases in natives can be dismissed in a few words, as they attended hospital as out-patients only, and only a single specimen of urine from each was examined. In the first case the urine contained a considerable amount of white deposit, consisting chiefly of pus cells and some epithelial scales. No red blood corpuscles were noted, but a few highly refractile, greenish-yellow cells resembling amoebae were found. These cells were about 20μ in diameter and were mostly spherical, but a few were observed to extrude hyaline pseudopodia clearly differentiated from the granular cytoplasm of the rest of the organisms. Movements were decidedly sluggish, and no ingested corpuscles were seen. The nucleus was clearly visible in most of the specimens.

The second case was more interesting. The patient complained of the passage of blood-stained urine, and the examination was made with a view to determining if he was suffering from Bilharziasis. The urine contained a great many red blood corpuscles, pus cells, squamous epithelial cells and Bilharzia ova, and in addition a considerable number of amoebae. The amoebae were actively motile, and had ingested a large number of red corpuscles. They had the appearance of *E. histolytica*, and measured about 20μ to 30μ in diameter. No cysts were found. Unfortunately the patient had already left the hospital before the specimen of urine was examined, and as he did not return for further treatment it was impossible to obtain any history of the disease or any further material for study.

We have been more fortunate with our third case, the European, and have been able to keep him under observation for over two months; and as his condition presented some points of interest, we propose to record it rather more fully. We have to thank Dr. C. V. Le Fanu for bringing this case to our notice, and for sending the

* Since writing the above, two more cases have been examined in which amoebae were present in the urine. The parasite was in each case *E. histolytica* (*tetragena*). Both the patients were male natives, and one was suffering simultaneously from urinary schistosomiasis.

patient to us with the diagnosis 'amoebae in the urine' already made. We are also indebted to him for enabling us to study the condition in detail, for his careful examinations which assisted so greatly in its elucidation, and for his valuable advice and help at every stage of our investigation. The history of the case which follows is moreover based largely on his notes, and we have pleasure in expressing our gratitude to him for permission to make use of them.

The patient, a European official, 27 years of age, first came under observation on 16th May, 1916, complaining of frequency of micturition and the passage of white deposit with his urine. He was a man of very fair complexion and good physique. His heart, lungs, spleen, and liver were normal, and the function of the bowels was natural. There was no fistula. In his habits he was active, and during 1914 used to cycle twelve to fifteen miles a day over rough roads. He was in his third tour of service in the Gold Coast (a tour consisting of twelve months' residence in West Africa followed by five months' leave), and had enjoyed fairly good health except for a 'short fever, probably malaria,' while on leave in July, 1915, and an attack of malaria early in 1916. He had never suffered from dysentery, but mentioned that he had had 'a slight diarrhoea in November, 1914, lasting for eight days.'

He gave no history of gonorrhoea prior to the end of 1914, but in December of that year, three days after connexion, he felt acute pain between the root of the penis and the anus which lasted for three days. There was no discharge, but, nevertheless, he used an injection of permanganate for one month. The patient evidently believed that he had suffered from gonorrhoea, but as the diagnosis was not confirmed by bacteriological examination, and as he never had a typical discharge, it is possible that the condition may have been of a different nature. When last in England on leave, some months after the onset of his illness, he was examined bacteriologically for gonorrhoea, but with a negative result, and we have recently made the same examination on several occasions without detecting gonococci.

He remained free from symptoms until May, 1916, when, following a slight accident, he felt pain in the left groin. A few days later frequency of micturition set in, the desire to pass water

being constant for twenty-four hours, and three days later he passed a large quantity of 'mucus,' after which his condition improved.

The urine when first examined, that is on 16th May, 1916, was a yellow colour, showed an acid reaction, and contained some white sediment in which Gram-negative bacilli, a little mucus, crystals of calcium oxalate, a few leucocytes and some epithelial scales were observed. There were also some spherical cells in the deposit which were highly refractile and greenish in colour which were probably amoebae, but as no signs of motility were seen, we did not feel certain about this. About a month later, on 14th June, the patient returned with a recurrence of his symptoms. His urine was now faintly alkaline (probably as the result of treatment), yellow, and loaded with a white flocculent precipitate. In the deposit, which consisted mainly of pus cells and epithelial scales, there were a few red blood corpuscles and a large number of the spherical greenish bodies previously observed. These cells had a highly granular appearance, and varied greatly in size. Most of them appeared to be quiescent or dead, their nuclei being distinct, but when carefully watched a few were seen to extrude clear hyaline pseudopodia. A few contained ingested red blood corpuscles. A more complete account of these bodies, which were evidently amoebae, will be given later. No casts were found in the urine.

On the following day, 15th June, the desire to micturate frequently had ceased, and the urine was pale yellow coloured, faintly acid in reaction, and almost absolutely clear. One or two white flakes were found in it after centrifugalisation, and in these pus cells and a very few amoebae were present.

The desire to micturate frequently returned on the evening of the 15th June, and turbid urine was passed. On the following morning the irritability persisted, and amoebae were found in abundance in the urinary sediment. On this occasion the urine was passed into two glasses, and it was observed that whereas the first part contained a considerable amount of deposit, the last part was practically clear. This observation suggested that the pus and the amoebae did not come from the bladder itself nor from the kidneys, but from some area further forward.

For the next day or two the patient passed normal urine, but on 20th June there was a slight return of his symptoms. On examina-

tion no amoebae could be found in washings from the anterior urethra, but after vigorous rectal massage a quantity of white lumpy deposit was obtained, and in this large numbers of amoebae were found. The deposit also contained immobile spermatozoa.

This attack has been described in detail, as it was apparently typical of those to which the patient was subject.

With the three-glass test, Dr. Le Fanu observed that most of the deposit was passed into the second glass, the third being almost free from it. On rectal examination, both seminal vesicles were found to be enlarged. The testicles and epididymis were normal, and there was no glandular enlargement in the groins. These observations pointed to a lesion of the genito-urinary tract in the neighbourhood of the seminal vesicles.

The treatment adopted consisted of repeated rectal massage of the vesiculae and daily hypodermic injections of emetine (.5 grs.). During the months of June and July he had diurnal frequency of micturition on two or three occasions, but was not troubled at night. The urine passed was of an extremely pale colour and abnormally low specific gravity. When last seen, on 7th August, the patient expressed himself as feeling much better, and his general condition was quite satisfactory. The urine on this occasion was very light coloured, slightly turbid, and contained some short 'threads.' It was neutral in reaction, had a specific gravity of 1008, and showed a slight cloudiness on heating, due to the presence of phosphates, but no albumen was present. The deposit contained inflammatory cells and an occasional amoeboid body. Treatment then appeared to have benefited the patient, but up to the time of writing had not completely cured him of his amoebic infection.*

THE MORPHOLOGY OF THE PARASITE FOUND IN THIS CASE

The morphology of the amoebae found in the urine of this patient was studied in freshly passed specimens and in fixed and stained preparations of the flaky deposit, and a brief account of the more important features observed is given below.

* The patient was completely cured by the third week in August.

Size. The amoebae varied greatly in size, but the majority were small. On one occasion, when the urine contained a large amount of white sediment in which innumerable amoebae were present, fifty individuals, taken as they came, were measured, and were found to average 10μ in diameter, ranging from 7μ to 33μ ; but more usually they averaged about 20μ in diameter, and some as large as 40μ were seen. Cysts were found, but none was seen containing more than four nuclei.

Shape. Most of the parasites, as seen in urine that had been passed some little time before examination, appeared to be in a quiescent condition, and were spherical. A few individuals were, however, pear-shaped, and others had thrust out pseudopodia which gave them an irregular outline. It was the presence of large spherical bodies of a slightly greenish colour that first drew our attention to the condition.

The Protoplasm. The appearance of the protoplasm varied, of course, with the age of the parasite, but there was always a distinct greenish-yellow tint and a conspicuously granular structure. In the spherical and quiescent individuals no distinction between ectoplasm and endoplasm could be made out, but in more irregularly-shaped parasites and in those exhibiting motility, it was clearly visible. The older amoebae were crowded with vacuoles, and the nucleus, which was usually easily seen, was often eccentric.

The Cytoplasm. The cytoplasm was composed of ectoplasm and endoplasm, but the distinction was not evident in the quiescent parasites. When the organism was moving, however, there was a distinct difference between the granular endoplasm and the hyaline glass-like ectoplasm of the pseudopodia; and in parasites of an oval or irregular shape even when not in motion, the same difference could be made out.

The endoplasm was granular, and contained a variety of ingested particles. A few of the parasites had ingested red blood corpuscles. Vacuoles were present in almost every amoeba, and were exceedingly numerous in the larger individuals. The vacuoles were not contractile.

The Nucleus. The nucleus was generally distinctly visible. It was large, and often situated somewhat eccentrically. Externally there was a well-defined nuclear membrane, refractile, and clearly

differentiated from the surrounding endoplasm. On the inner side of this membrane there was usually a large amount of chromatin arranged in irregular nodular masses, and throughout the nuclear substance similar chromatic matter was distributed. The karyosome was large, and showed a centriole.

Vacuoles, &c. Vacuoles were almost always present, and were generally very numerous. They were not contractile. The parasites were evidently actively phagocytic, and had ingested a variety of bodies, including erythrocytes.

Motility. The majority of the amoebae seen in the urine were quiescent, but a few when watched carefully were seen to extrude pseudopodia. The movements were sluggish, and did not result in active progression. The pseudopodia were blunt processes of ectoplasm with a clear glass-like appearance, and contained neither granules nor vacuoles.

Stained preparations. With haematoxylin or Romanovsky methods, or simply with methylene blue, the parasites stained well, but intensely. When taken directly from the urine, and stained in the ordinary way by the Romanovsky method (Giemsa), the whole organism was coloured a dark purplish hue, in which the nuclei could just be distinguished as almost black masses and the larger vacuoles as pink or purple discs; but after careful washing in salt solution the reaction became normal, the cytoplasm staining blue and the nuclei red. The action of the urine was evidently responsible for the peculiarities first observed, a conclusion that was confirmed by immersing in urine active amoebae (*E. histolytica*) from the faeces of a dysenteric patient, and finding that the same changes in staining reactions were produced.

In well stained specimens there was no characteristic difference between the reactions of the ectoplasm and endoplasm. The cytoplasm was highly vacuolated, and contained a number of food particles, &c., but although the urine was full of bacteria they did not appear to have been taken up by the amoebae to any considerable extent. Red blood corpuscles in various stages of digestion occurred in some of the parasites.

The structure of the nuclei varied a good deal, but in the majority of the amoebae it was of the *Entamoeba tetragena* type, and consisted of a centriole with a clear area round it, bounded by

the outer border of the karyosome, on which small particles of chromatin were collected. Outside this a reticulated zone reaching to the nuclear membrane, on which there was, as a rule, a considerable quantity of chromatin. Many of the variations observed were probably due to the changes occurring during the 'cycle of the karyosome' described by Hartmann, which we were able to follow in our preparations, but others may have been degenerative, and the result of the action of the urine on the organisms. The nuclear structure of *E. tetragena* has been so thoroughly studied, and is so well known, that it will be unnecessary to enter into greater detail here. Suffice it to say that most of the amoebae showed a nucleus of the *E. tetragena* type, and that such variations as were seen were an approximation, more or less complete, to the *E. histolytica* type.

As we have already explained, the kidneys and the bladder did not appear to be affected in this case, and we believe we were able to localise the lesion in the neighbourhood of the seminal vesicles. This fact must be taken into account in considering the characters of the amoebae, for when examined they were immersed in the urine, which was probably highly injurious to them. It is probable, indeed, that most of the parasites were already dead before they were examined, and that this explains the clear definition of the nucleus and the absence of motility in most of the cells. Those that were not actually killed were probably profoundly affected, and therefore exhibited only a slight degree of rather sluggish movement. It was actually demonstrated by taking active amoebae (*E. histolytica*) from the faeces of a dysenteric patient and immersing them in urine that changes were produced similar to those observed in this case.

Making all due allowance for the pernicious action of the urine, and considering the morphology of the parasites, their nuclear structure and the occurrence of cysts with four, but never more than four, nuclei, we believe that these amoebae cannot be differentiated from *Entamoeba histolytica* (*tetragena*).

THE PROBABLE MODE OF INFECTION

The patient, as has been stated earlier, had never suffered from dysentery so far as he was aware, and at the time he came under our observation his faeces contained neither amoebae nor amoebic cysts; but about a month before his illness commenced he had a slight attack of diarrhoea, which lasted about eight days. This responded to simple treatment, and he thought nothing of it at the time. It is well known, however, that the remote complications of amoebic dysentery tend to occur 'months, or even years, after the primary dysenteric attack, which may have been a very slight one, so that the connexion with it is quite liable to be overlooked.'

Referring to amoebic hepatitis, Rogers (1913) points out that it 'not rarely occurs in subjects who give no history of ever having suffered from actual dysentery,' and if this is true of one of the remote complications of amoebic colitis, it is probably true of others. We are, therefore, inclined to believe that this patient may at some time have unconsciously harboured amoebae in his large intestine, and that they may have found their way thence, either directly or indirectly, to the neighbourhood of the seminal vesicles, setting up an inflammatory process ending in suppuration and the discharge of pus containing amoebae through the urethra.

GENERAL CONSIDERATIONS

In the literature at our disposal, we have been able to find references, more or less complete, to about a dozen cases of urinary amoebiasis in addition to the three we have ourselves observed, and it may be of interest to consider them briefly.

In the majority of the patients the infection has been with *Entamoeba histolytica* (*tetragena*). This identification was definitely made by Craig, Fischer, and Lynn, and we have come to the same conclusion with regard to the parasite in our cases. The earlier records, those of Baelz, Jürgens, Posner, and Kartulis, are also generally believed to have referred to the same species. The morphology of the amoeba in the case recorded by Ward, Coles and

Friel has not yet been adequately described, and no identification can be made at present; but they have hitherto mentioned no characteristic that I have not observed in specimens of *E. histolytica* taken from the faeces after immersion in urine. It is possible that all the cases of urinary amoebiasis recorded may have been caused by *E. histolytica* (*tetragena*).

If the amoebae are not actually living in the urinary tract, but are in some neighbouring tissue from which they are discharged into the bladder and urethra, they may be greatly altered in appearance by contact with the urine, and may actually be dead before they are passed. This fact must not be forgotten in studying the morphology of the organisms, as they may be rendered unfamiliar-looking and difficult to identify.

Although the parasite may have been the same in all the cases, the site of the lesion has varied. In Posner's case there were casts in the urine, and the kidneys seemed undoubtedly to have been affected. In several other cases the lesion was equally certainly in the bladder. This was so in the original case, that of Baelz, in Jürgens' patient who had small mucous cysts containing amoebae in the bladder, and in Kartulis' subject who had a vesicular tumour. Craig's case stands on a different plane, since a sinus was discovered at the autopsy which connected the bladder with the ulcerated intestine. In one of our cases, the patient suffering simultaneously from Bilharziosis, the infection was probably in the bladder. Cystitis, or some condition interfering with the proper function of the bladder, seems to have been present in all these cases, and it may be that the altered composition of the urine accompanying these conditions enabled the amoebae to lodge in this organ. Fischer's case is somewhat obscure, but the urine was very acid, so that it seems probable that the parasites were not located in the bladder itself. Lynn's case, in which there was a burning sensation on micturating, and which was complicated by a prostatic stricture, may have been due to a lesion in the urethra. Our case, in a European, is the only one of the series in which the lesion was definitely localised in the neighbourhood of the urethra. We have stated our reasons for believing that the amoebae were lodged in the vicinity of the seminal vesicles, and if we are correct in our interpretation of the facts the condition was rather a remote

complication than a direct amoebic infection, and the presence of the organisms in the urine was in a sense accidental.

The genito-urinary tract is liable to amoebic infection by several routes. The parasites may gain access directly either from the outside by way of the urethra or from an ulcerated intestine through the intermediate tissues, and indirectly from the bowel through the blood stream. In Baelz's case, the vagina was infected as well as the bladder, and he believed that the parasites had been introduced with water used for washing the parts. The vagina was also infected in Jürgens' patient, and the amoebae may have spread thence to the bladder. Craig's case was an example of the direct spread through the tissues from an ulcerated intestine. Our European patient may have obtained his infection from the intestine either directly, or more probably indirectly, through the blood.

As regards sex and age incidence, no conclusions can be drawn from so small a number of cases, especially as several of them occurred in countries where the majority of the patients seen would be adult males. Direct infection by way of the urethra, however, might be expected to be more common in women, and this does appear to have been the case.

Urinary amoebiasis may be due to a primary infection or secondary to amoebic dysentery. It would certainly seem that cystitis, tumour of the bladder, and gonorrhoea are predisposing causes.

ACCRA,

August, 1916.

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THE MORPHOLOGY OF CERTAIN SPIROCHAETES OF MAN AND OTHER ANIMALS

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I. INTRODUCTION

ON THE MEASUREMENT OF THE LENGTHS OF SPIROCHAETES

It has been recognised for many years that it is extremely difficult to distinguish the smaller spirochaetes by morphological characters. Some authors have gone so far as to assert that 'morphology does not constitute a valid test of species,' and there are a great number of references in the literature to the variations of these organisms.

The discovery of the fact that spirochaetes can be cultivated, although it has placed additional means of identification in the hands of the protozoologist, has not disposed of the difficulty, for the technique is often difficult and laborious, and not all spirochaetes can be grown in artificial media. For diagnostic purposes a morphological criterion is still urgently needed.

There is as a rule nothing distinctive about the movements of the

smaller spirochaetes. All those that I have examined have exhibited at some time or under some circumstances all the types of motion considered characteristic of these organisms. But as the movements vary with the size and condition of the individual spirochaetes, the length of time they have been removed from the body, the temperature, the chemical composition of the fluid in which they are immersed, and other circumstances, they could never be of much value in classification.

Staining reactions are equally unreliable, as they are inconstant, and in the same organism can be made to vary by changing the composition of the medium even very slightly. The structural characters that can be made out in such small parasites are not of much assistance either, since the majority have more or less sharp ends, delicate sinuous bodies which may or may not show some indications of a membrane, and which are sometimes banded by chromatin rodlets and sometimes contain coccoid granules. A decided tendency to form tangles such as occur at the crisis of the diseases caused by some blood spirochaetes, and a definite intracellular phase when it occurs, may, however, be of some assistance. The methods of division, whether longitudinal or transverse, cannot be relied upon, since in many species both occur either simultaneously or under different conditions of growth.

Much importance was at one time attributed to the number and the size of the waves into which the body was bent, but it is now known that these are subject to great variation, depending on a number of factors, such as the size and thickness of the individual organism, the nature and rapidity of its movements, the reaction of the medium, and so forth.

The thickness of spirochaetes also varies considerably in different individuals of the same species, and is very difficult to measure accurately. In the living organism it can only be guessed, and in permanent preparations it varies to some extent with the fixative and the stain employed.

Length is perhaps the morphological character which promises to be of greatest assistance in identification, notwithstanding the fact that considerable variations are known to occur. The length of a species of spirochaete is usually stated as ranging from a minimum to a maximum, but this is unsatisfactory and often actually

misleading, since it affords no means of distinguishing organisms of approximately the same size, and gives no indication of the most common form of the parasite. As I have pointed out elsewhere, this method should logically include the minutest spirochaete just developing out of a coccoid granule and the longest multiple form found under any conditions.

A more reliable and more helpful method of ascertaining the length would be, I believe, to draw with the aid of a camera lucida a large number of the spirochaetes taken as they came, to measure the drawings either by the tangent line or compass method, and to plot the lengths as a curve. As a rule, it would be sufficient to measure thus two or three hundred individuals, but occasionally it might be necessary to draw a larger number taken at regular intervals during the course of the infection. This method would be similar to that adopted in the case of trypanosomes.

In the case of the spirochaetes described briefly in the ensuing pages, this method of determining the length of the organisms has been adopted, and I have employed the same method in the cases of two other spirochaetes described elsewhere (see Table I). The method shows, I think, that although considerable variations do occur, the great majority of the spirochaetes of the same species measure within a few microns of each other in length. The measurements, according to length, may be grouped in the following manner. First there come a few very short forms, which are probably either abnormally small individuals or, in the case of spirochaetes possessing an intracellular phase, immature specimens that have been prematurely freed in the process of making the films. Then there come the normal single spirochaetes which form the dome of the curve and extend over a range of three or four microns. In this group are included all forms, from the recently separated daughter spirochaetes to the fully developed single individuals. This is, in my opinion, the characteristic length of the organism. Next come the pre-division forms and the forms already differentiated into two daughter spirochaetes, united end to end by a delicate filament; and at this point on the curve a small subsidiary crest or halt in the descent may be observed, due to the overlapping in length of large single and small double forms. Finally there come the abnormally long individuals, including hypertrophic specimens,

TABLE I.--The distribution according to lengths, by percentages, of certain spirochaetes of Man and other animals.

[illegible]

unusually large dividing forms, incompletely divided organisms, and the multiple forms not infrequently met with in some cases.

Besides giving a clear idea of the natural size of the organism, this method of plotting the measurements of length may assist in the recognition of mixed infections. When but a single species is present the curve rises rapidly to a crest and rapidly declines, and a short range of three or four microns includes the majority of the individuals. In the case of a mixed infection the curve both rises and falls more gradually, and the range of measurements including the majority of the organisms, is longer. As an illustration of this difference in the form of the curve, the measurements of two hundred spirochaetes from human faeces, *S. eurygyrata*, and a similar number from the human throat are plotted by percentages as curves separately, and combined so as to represent a mixed infection with these two organisms in equal proportions (see Table II and Chart I).

In each of the separate curves there is a well-marked crest, and the commonest lengths, namely, those including 10 per cent. of the organisms, or more, cover a range of 4 microns, from 3μ to 6μ in the case of *S. eurygyrata*, and from 6μ to 9μ in the case of the throat spirochaete; but in the combined curve the crest is less marked, and the commonest lengths extend over a greater range.

TABLE II.—The distribution according to length of 200 spirochaetes from human faeces, and a similar number from a human throat.

Site of infection	LENGTHS IN MICRONS																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Faeces ...	2	38	57	55	31	10	4	2	...	1
Throat	1	3	15	29	36	40	26	15	13	12	3	3	2	1	...	1
Totals ...	2	39	60	70	60	46	44	28	15	14	12	3	3	2	1	...	1
Percentages ...	0.5	9.75	15.0	17.5	15.0	11.5	11.0	7.0	3.75	3.5	3.0	0.75	0.75	0.5	0.25	...	0.25

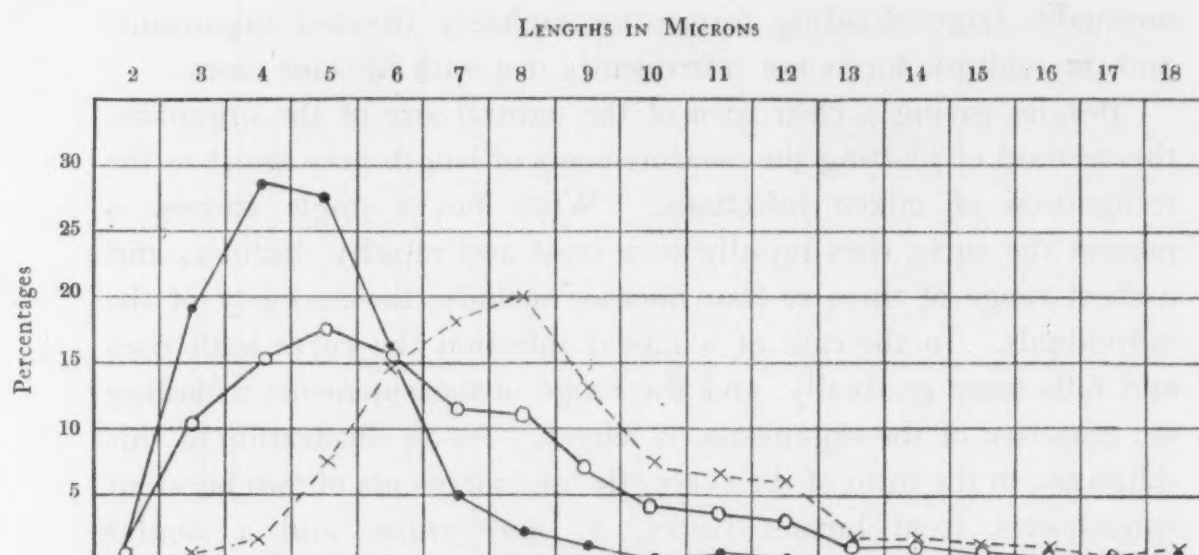


CHART I.—The distribution, by percentages, according to length of spirochaetes from the human faeces (● —● —) and throat (× — — × — —), and the curve representing a mixed infection with these two organisms in equal proportions (○ —○ —).

II. A SPIROCHAETE FOUND IN THE VAGINA

It might be expected that spirochaetes would be frequently found in the vagina since the conditions in this locality must often be favourable for their growth. So far as I am able to ascertain, this is not the case, and with the exception of *Spirochaeta aboriginalis* associated with ulcerative granuloma of the pudenda, and *Spirochaeta phagedenis* found in an indurated and ulcerated swelling of the labium, no organisms of this nature have been described from the vagina. It may, therefore, be of interest to record briefly a case of vaginitis, which has recently come under my notice, in the discharge from which spirochaetes were present, which, so far as could be judged, appeared to be the cause of the disease.

CLINICAL CONDITION AND THE MATERIALS EXAMINED

The patient was a girl, seventeen years of age, a native of Lagos, who came to the Accra Hospital complaining of discharge from the vagina. On examination, the surface of the vagina was found to be slightly red and congested, but there was no ulceration. The discharge was scanty. Three dried smears of this discharge

were sent to the laboratory for examination, and in them a large number of spirochaetes were found, together with some long Gram-positive bacilli and numerous epithelial scales. Unfortunately, the patient had already left the hospital before the specimens were examined, and she did not return for further treatment. The living spirochaetes could not therefore be examined.

MORPHOLOGY OF THE SPIROCHAETES

The morphology of the spirochaetes was studied in the three dried films of the discharge, after staining with Romanowsky (Leishman), gentian violet, and by Gram's method, using fuchsine as the counter stain. The parasites stained well with these reagents, were decolourised by Gram's method, and with Leishman's stain were coloured a purplish tint.

The spirochaetes showed a considerable degree of morphological variation, but always had flexible sinuous bodies, bent into a number of curves or waves.

The number of waves was very variable, and this was probably due to the nature of the movements of the organisms at the time when they were fixed. Some had practically no coils, or only a few large flexions, giving them a contorted appearance (fig. I, 4-8).

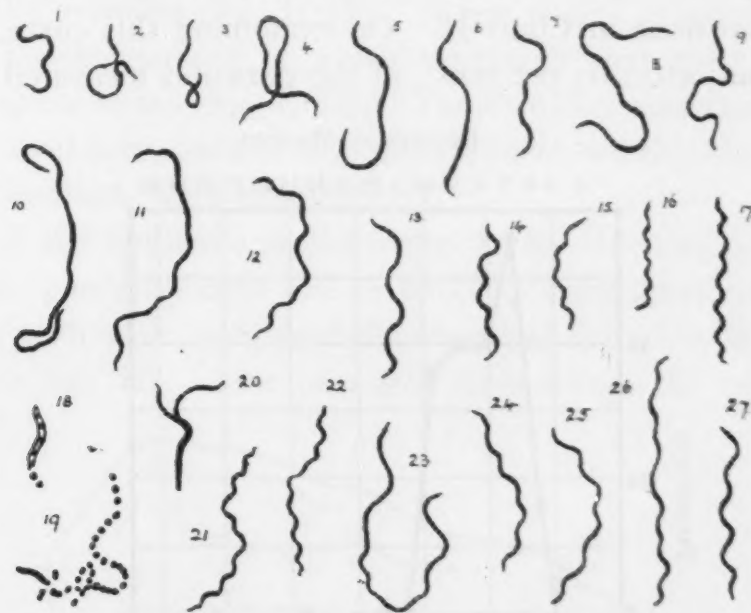


FIG. I.—*Spirochaeta vaginalis*, Macfie. 1-27: various forms found in the vaginal discharge; 18: coccoid bodies escaping from the periplastic sheath; 19: coccoid bodies apparently formed from spirochaetes; 20: a parasite undergoing longitudinal division; 21-23: others undergoing transverse fission. All the figures were drawn with the aid of an Abbé-Zeiss camera lucida at a magnification of 2000 diameters.

These spirochaetes had probably been executing lashing movements and undulatory flexions at the moment of fixation. Others showed a large number of small close waves such as are associated with rapid helicoid movements (fig. I, 14-17). In some the waves were deep, in others they were shallow; and the number varied also in relation to the size and thickness of the organism. No general statement as to the number of waves characteristic of the organism can be made, but sometimes there were as few as two and sometimes as many as nine or ten. The number of coils is probably not important, and, as Fantham (1916) has pointed out, cannot be regarded as a specific character.

The ends of the spirochaetes were tapering, but some were more pointed than others. The organisms were extremely slender, so that it was not possible to measure them exactly, but the majority were about 0.2μ broad. There were, however, considerable variations, some individuals being obviously thicker than others.

The variation in length of the spirochaete was determined by drawing a hundred individuals taken as they came, with the aid of a camera lucida, and measuring them. The shortest spirochaete measured was 4μ , the longest 20μ , and the average length worked out at 9.3μ (see Table I). A better idea of the length characteristic of the organism is, however, obtained by plotting the measurements, as has been done in Chart II. On examining this curve, it is clear that the majority, 63 per cent., of the parasites measured 7μ to 10μ

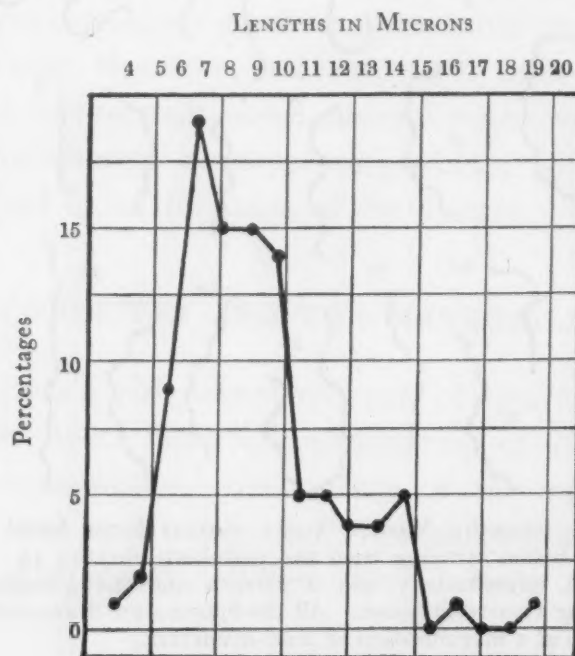


CHART II.—The distribution according to length of *Spirochaeta vaginalis* (Macfie).

in length. Although the number of measurements was small, it is evident from the nature of the curve that only a single species of spirochaete was being dealt with, and one whose length was most commonly 7μ to 10μ ; it was therefore considered unnecessary to measure a larger number. The slight secondary crest on the curve at 15μ was no doubt due to the overlapping in length of large single and small double forms.

So far as could be judged from the materials available, division seemed to occur most commonly by the transverse method, organisms compounded of two daughter spirochaetes linked by a delicate filament being frequently seen (fig. I, 21 and 22). A few Y-shaped parasites were, however, present which appeared to represent a stage in longitudinal division (fig. I, 20). The common stem of these forms did not show any signs of being double, and was quite unlike the re-curved forms also occasionally seen in the smears.

The cytoplasm of the majority of the spirochaetes appeared to be homogeneous, but a few showed very beautifully a succession of granules or rodlets at short intervals along their bodies. No vacuoles could be distinguished. Slight indications of a sinuous membrane were seen in one or two specimens only.

Coccoid bodies were seen within some of the spirochaetes, and in others the end of the organism appeared to have disintegrated, liberating the granules (fig. I, 18). A few chains of granules were also seen, the appearance of which suggested that they had been formed in spirochaetes (fig. I, 19). These chains were composed of rounded or oval bodies resembling minute cocci, which, when stained by Gram's method, were decolourised.

A few of the epithelial scales found in the discharge contained spirochaetes, generally only one or two, but occasionally more. A part of one such scale is figured showing five spirochaetes and two bacteria (see fig. II). The parasites seen within the scales were



FIG. II.—Portion of an epithelial scale from the discharge in which five spirochaetes and two bacteria are shown. Drawn with the aid of an Abbé-Zeiss camera lucida at a magnification of 2000 diameters.

generally small and of a simple form, and suggested the possibility that this organism passes through an intracellular phase similar to that which I have described as occurring in *S. urethrae*. Unfortunately the materials available were insufficient, and the infection at the time of examination was not heavy enough to have enabled me to follow out such a phase through all its stages, even supposing that it did occur.

THE IDENTITY OF THE SPIROCHAETES

In ulcerative granuloma of the pudenda a spirochaete, *S. aboriginalis*, has been found which measured about 12μ in length, ranging from forms a few microns to others 18μ or 20μ long, and had irregular waves. Another spirochaete, *S. phagedenis*, was found by Noguchi in an ulcerated swelling of the labium, and this organism measured from 4μ to 30μ in length. The patient from whom the spirochaete described above was obtained suffered from a slight vaginitis, but had no ulcerative lesions, and even apart from this the parasite she harboured differed in dimensions from both *S. aboriginalis* and *S. phagedenis*.

Treponema pallidum might under certain circumstances be found in the vagina even at a time when no specific lesions were visible. The spirochaetes found in this case sometimes had a membrane and the coils were not fixed, so that this organism can be excluded. *Spirochaeta refringens* or *S. balanitidis* might be introduced into the vagina, and if the conditions were favourable might continue to multiply there. These two organisms are considered by some authorities to be the same, and as they are generally described as measuring 8μ to 12μ in length, they are probably distinct from the spirochaete found in this case. Some writers state that *S. refringens* measures 20μ to 35μ in length.

The ill-defined *S. vincenti* might conceivably invade the vagina, but this species is also a somewhat larger organism, measuring as a rule 12μ to 25μ in length, and sometimes attaining 40μ . *S. urethrae*, the organism I described recently from a case of urethritis, measures most commonly 8μ to 12μ in length, and is a slightly larger parasite.

Spirochaetes from the intestine might find their way into the

vagina. A number of different species have been described as occurring in the human intestine, such as *S. hachaizae*, *S. eurygyrata*, *S. stenogyrata*, and others, but Fantham (1916) has recently shown that all these parasites are morphological variations of a single species, *S. eurygyrata* Werner emend. Fantham. This organism he has defined as having tapering ends, and measuring 3μ to 15μ in length, and about 0.25μ in breadth. *S. eurygyrata* is therefore a much smaller organism than the one found in the vagina of this native girl.

It must be concluded that the vaginal spirochaete described above is distinct from all these organisms, and I therefore propose for it the name *Spirochaeta vaginalis*.

CONCLUSIONS

1. Spirochaetes were found in the discharge from a mild case of vaginitis in a native woman which it is believed were the causal agent of the disease.
2. These organisms, which had pointed ends, were most commonly 7μ to 10μ in length, but of a hundred individuals measured the longest was 20μ and the shortest 4μ long. They were about 0.2μ in breadth, and might show any number of waves from two to ten.
3. The name *Spirochaeta vaginalis* is proposed for this parasite.

III. A SPIROCHAETE FOUND INHABITING THE BLADDER

In a case of chronic cystitis large numbers of spirochaetes were found in the urine, which, although they were probably not the cause of the disease, are of interest because of the extraordinary degree of morphological variation they showed.

The patient, a native man, 30 years of age, stated that three years ago he contracted gonorrhoea, that as a result of this infection an obstruction formed in his urethra causing the penis to swell greatly, and eventually, about a year ago, to burst. He came to hospital in order to undergo an operation because he was passing his

urine through two fistulae, one on either side of the penis, about two inches from its end. The urethra was completely occluded by a stricture just in front of these fistulae. The patient complained also of pain over the region of the bladder, and there was a little white discharge from the fistulae.

The urine was brown in colour, turbid, strongly alkaline, and had a specific gravity of 1015. Albumen, pus, and a trace of blood were present, but no sugar. On standing, an abundant sediment collected at the bottom of the glass, which was composed mainly of triple phosphates crystals, but contained also a large number of pus cells, a few red blood corpuscles, epithelial scales, yeast cells, bacteria, and a considerable number of spirochaetes. The bacteria were countless, and included staphylococci, streptococci, motile and non-motile Gram-negative and Gram-positive bacilli. No casts were found.

The pus taken from the urethral fistulae contained the usual polymorphonuclear cells, epithelial scales, staphylococci, and Gram-negative and Gram-positive bacilli. With the exception of a few clumps of bacteria, there were, however, very few organisms present, and it was only after careful search that any spirochaetes were found. It was evident, therefore, that the main infection was in the bladder, and that the spirochaetes were living chiefly, if not solely, in the urine. No gonococci were found in either the urinary deposit or the discharge from the fistulae.

From the clinical aspect, the case was therefore one of chronic cystitis secondary to a urethral infection, and there was no reason to regard the spirochaetes found as anything more than saprophytic organisms living and multiplying in the pathological urine in company with the hosts of bacteria.

THE CHARACTERISTICS OF THE LIVING SPIROCHAETES

On examining the urine two apparently distinct types of spirochaetes were seen, both occurring in considerable numbers.

The first type was a small, slender organism that moved rapidly about the field of the microscope. Its movements were mainly composed of undulatory flexions and corkscrew motions, but sometimes the organism showed active lashing movements.

Progression might be in either direction, and a reversal of the direction was sometimes witnessed. The body of the spirochaete was slender, the ends appeared to be pointed, granules in the cytoplasm although sometimes present were not conspicuous, and there were usually relatively few waves. This type closely resembled the *S. eurygyrata* so commonly found in the faeces in this country.

The second type was a long, sluggish organism. Many of the spirochaetes of this type were quite motionless, and those that were active showed only slow undulant and helicoid movements. Progression was deliberate, but might take place in either direction, and a reversal was seen several times. These organisms were decidedly broader than those of the first type, their ends were usually quite blunt, and their bodies were conspicuously granular and often beautifully banded. The relatively enormous length of some of these spirochaetes was perhaps their most remarkable feature. As a rule, the body was bent into a large number of waves, sometimes as many as twenty-six, and this was just as noticeable in the immobile individuals as in those exhibiting movements. Many of these organisms looked as if they were made up of two or more parts, but although a number of them were watched carefully, division was never observed. It should perhaps be stated at once that the possibility that these forms might be bacterial was not overlooked, but that after careful consideration it was dismissed.

Two more dissimilar organisms than these could hardly be imagined; the one short and most active, the other very long and very sluggish.

MORPHOLOGY

The spirochaetes of both types showed a high degree of morphological variations. The organisms of the first type (fig. III, 1-28) had slender flexible bodies bent into a number of waves or coils. The waves were generally two to five in number, but some of the spirochaetes were simply bow-shaped and others had more than five. The ends of the organism were tapering. The cytoplasm of the body was usually homogeneous, but some of the larger individuals showed transverse chromatin bands or rods, and others contained granules. A membrane was never seen distinctly, but some specimens appeared to have a band partly filling up the trough

of some of their waves, which may have been this structure. The spirochaetes were very slender, so that it was difficult to measure them, but their thickness was about 0.25μ . Some individuals were

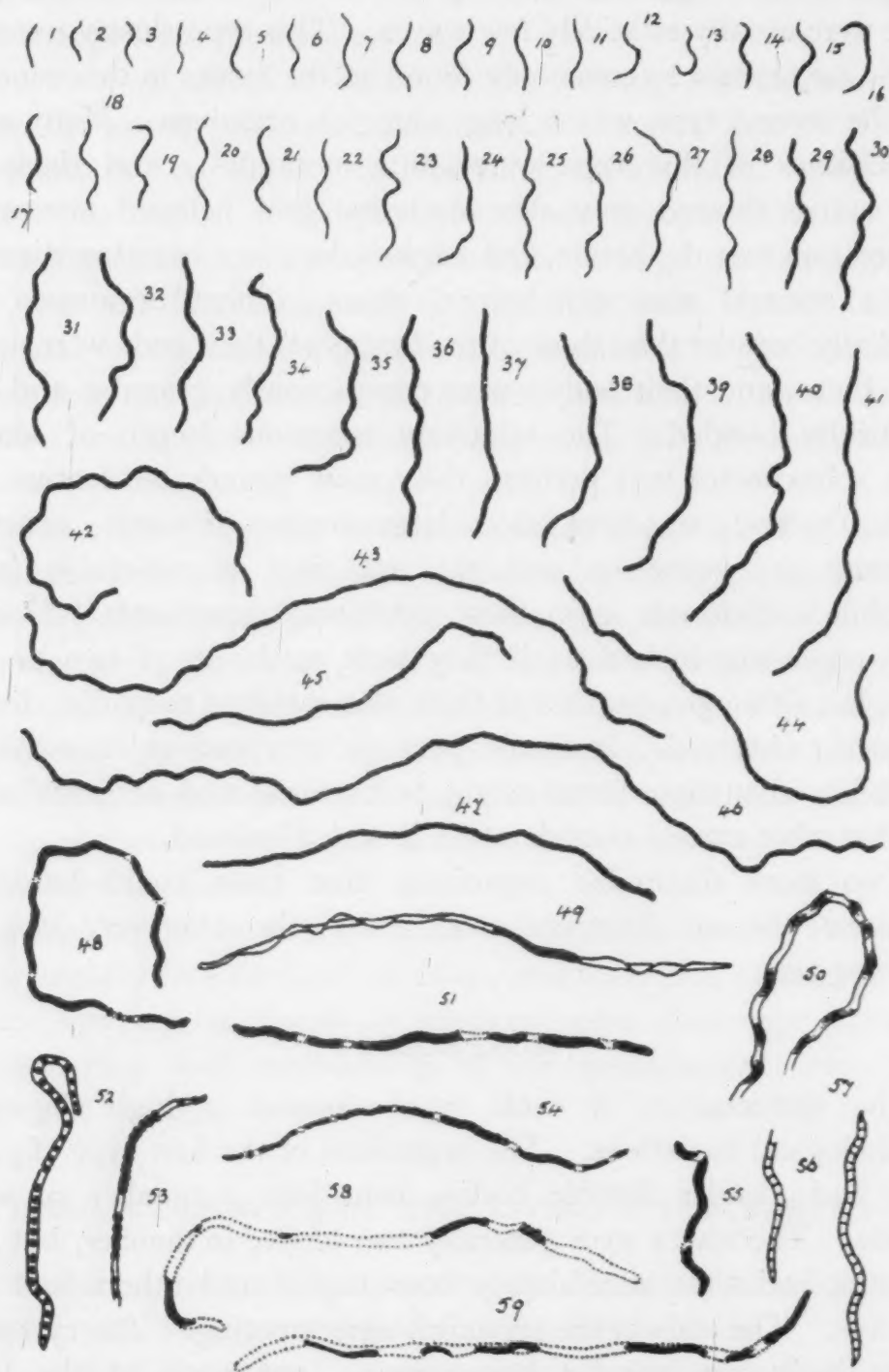


FIG. III.—Various forms of spirochaetes found in the urine in a case of chronic cystitis. All the drawings were made with the aid of an Abbé-Zeiss camera lucida at a magnification of 2000 diameters; the thickness of the organisms, however, is only approximately indicated, and in 48-59 it has been exaggerated somewhat in order to show the structure. 50, 53, and 56 show parts of longer spirochaetes.

decidedly stouter than others. When stained by Leishman's method the organisms were usually coloured purplish, but this is not really a matter of any importance, as the tint assumed by spirochaetes can be made to vary by changing the chemical constituents of the fluid in which they are living.

A typical spirochaete of the second type was a much coarser organism, and there was the greatest variation in size and shape and structure (fig. III, 29-57). The body was bent into waves, which were sometimes well marked and at other times indistinct, and also long undulations on which the waves were superimposed. Small specimens might show only three or four waves, but large ones, as has already been mentioned, might have as many as twenty-six. The ends of the spirochaetes were generally distinctly blunt, but they often tapered somewhat, but were never so sharply pointed as those of the first type. A periplastic membrane was sometimes visible, but reference will be made to this again later. The organisms were broader than those of the first type, and measured usually 0.4μ in thickness. The breadth did not sensibly increase with the length of the body, the extremely long individuals being just about the same thickness as the shorter ones. There were, however, considerable variations, and some long specimens were no broader than the spirochaetes of the first type. The structure of the cytoplasm varied a great deal. Some of the spirochaetes, and these were generally the smaller ones, appeared to be almost homogeneous; but more usually the body stained irregularly. Chromatin bars or rodlets at short intervals all along the body were sometimes seen most clearly (fig. III, 56 and 57), and other organisms seemed to have broken up into rounded or oval granules within the periplastic sheath (fig. III, 52). Most of the larger individuals, however, were composed of a sheath in which irregularly shaped segments of various sizes were enclosed. When stained by Leishman's method the sheath was coloured grey or dull blue, and the granules a chromatin tint. In some specimens the granules appeared as rather diffuse collections of chromatic material, more concentrated in the centre, and at the periphery merging gradually into the general cytoplasm (fig. III, 50); in others the granules were shaped like narrow rods, which when carefully examined were seen to be beaded (fig. III, 53); but most commonly the periplastic sheath contained a

larger or smaller number of homogeneous bodies, rounded, bacilli-form, or shaped like spirilla (fig. III, 48, 51, 54). From some of the spirochaetes nearly all these bodies seemed to have vanished, leaving an empty periplastic sheath (fig. III, 49 and 58). It was in these specimens that the appearance of a membrane was most distinct, but the effect may have been due to the irregular twisting of the sheath or to damage done at the time when the enclosed bodies escaped (fig. III, 49).

In addition to these two distinct types of the organism, there were a number of intermediate forms which bridged the gap both in length and breadth.

As regards the length of the spirochaetes found in this case, two hundred individuals, taken as they came, were drawn with the aid of a camera lucida and measured. The shortest specimen measured 2μ , and the longest 66μ . The latter measurement was not the maximum, as elsewhere in the films even longer individuals were found. It was at once obvious that to take the average of such measurements would be highly misleading, and, as a matter of fact, this average was 11μ , a length to which only 3 per cent. of the spirochaetes belonged. The measurements were, therefore, distributed according to their lengths in microns, and it was hoped that in this way some indication might be obtained as to the nature of the infection. As the curve formed by these measurements (see Chart III) appeared to be satisfactory, it was considered unnecessary to measure a larger number of the organisms.

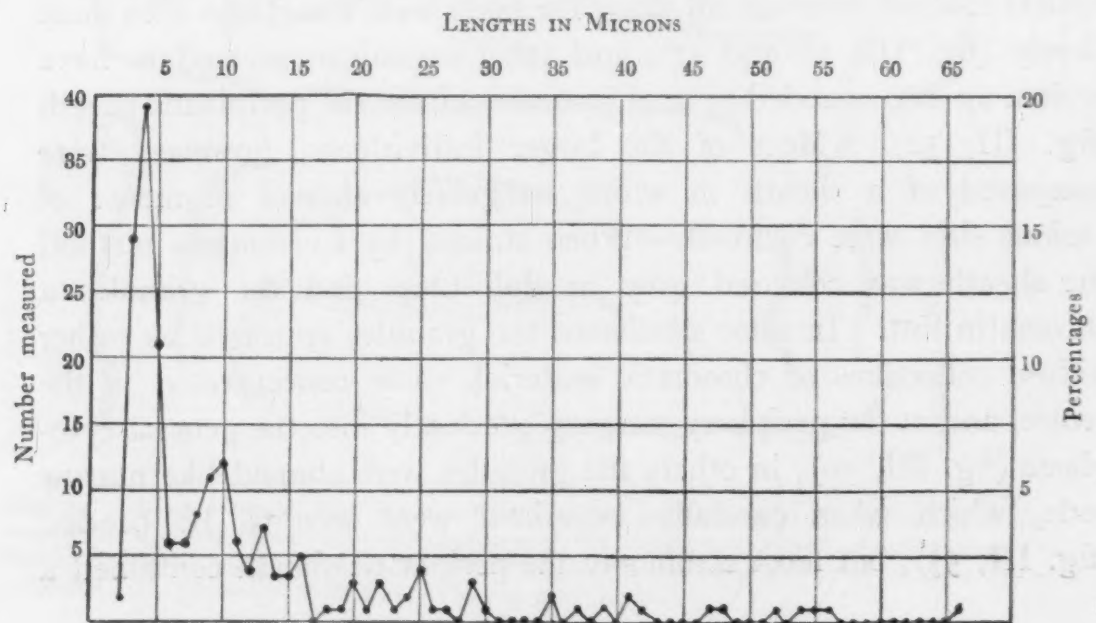


CHART III.—The distribution according to length of the spirochaetes found in the urine in a case of chronic cystitis.

An examination of this curve is, I think, instructive. It shows in the first place a most pronounced crest, which proves, I believe, that the spirochaetes all belonged to a single species. This species measured from 2μ to 16μ in length, and one must conclude that all the longer specimens were multiple forms, a view that is supported by their morphology. The greatest number of the spirochaetes, nearly 20 per cent., measured 4μ , and 44.5 per cent. of them were within 3μ of each other in length, between 3μ and 5μ . This is the most characteristic length of the species. The curve shows a secondary crest at 9μ to 10μ , indicating the lengths at which large single and small double forms overlap. The length of the spirochaete may be said, therefore, to vary from 2μ to about 10μ , but to be most commonly 3μ to 5μ long.

THE IDENTIFICATION OF THE SPIROCHAETE

The curve formed by distributing the measurements of length of the two hundred spirochaetes drawn indicates that the small type of the organism was the true form of the parasite; and this organism appeared to correspond with the descriptions of *S. eurygyrata*.

S. eurygyrata was originally described by Werner (1909) from specimens found in his own stools, and was stated to be 'a wide wound form' measuring from 4.6μ to 7.3μ in length. The same author found in the same materials 'a narrow wound form' varying in length from 3.5μ to 6.1μ , which he considered to be a distinct species, and which he named *S. stenogyrata*. Fantham (1916) has recently studied the spirochaetes occurring in human faeces, and has come to the conclusion that both the parasites described by Werner, and several others briefly referred to by earlier writers, are morphological variations of the same species. *S. eurygyrata*, Werner emend. Fantham, is defined as an organism which has pointed ends, measures from 3μ to 15μ in length, and is about 0.25μ in breadth. These measurements are almost exactly those which I determined as being characteristic of the spirochaete found in the urine of the case described; and a comparison of the morphology of the organism I have described with that of *S. eurygyrata* given by Fantham can leave no doubt as to the identity of the two organisms.

The patient whose case I have recorded was suffering from a

chronic cystitis, and the history of his illness was such as to make it highly probable that organisms from the rectum might have invaded the bladder, and it is probable that the spirochaetes found multiplying in the urine had originally been derived from this source.

THE SIGNIFICANCE OF THE LONG TYPE OF SPIROCHAETE

The curve shown in Chart III suggests that the long type of the spirochaete was a multiple form, since those measured were found to be distributed irregularly over a wide range of lengths.

In the urine of the patient, as is usual in chronic cystitis, there were a great number of epithelial scales, but neither in living specimens nor in fixed and stained preparations were any spirochaetes seen in these cells. The spirochaetes, in fact, appeared to be saprophytes, and the urine in the bladder, so far as could be ascertained, was just a culture of these organisms in symbiosis with a varied bacterial flora. The long type of the spirochaete was probably comparable to the multiple forms frequently found in cultures of these organisms.

The smallest individuals of the long type were perhaps hypertrophic, as they sometimes had almost homogeneous cytoplasm or showed a banding of their bodies by chromatic rods (fig. III, 55-57). Slightly larger specimens were frequently seen to contain coccoid bodies, often arranged in rather irregular groups (fig. III, 53). The very long organisms, however, were almost always composed of a delicate periplastic sheath, within which a number of deeply stained bodies were enclosed (fig. III, 51, 54). These bodies were homogeneous in structure and varied in size and shape, some being rounded or oval, some bacilliform, and others bent or curved like spirilla. The latter bodies resembled in shape and size the small free forms of *S. eurygyrata*, but differed from them in having blunter ends. The appearance of these long spirochaetes was such as might be expected to result if the coccoid bodies, instead of being liberated from the organism, were to develop within the periplastic sheath. It is possible that some of the very long individuals had been formed in this manner, but others were probably true multiple forms in which transverse division was incomplete, leaving the daughter spirochaetes still attached end to end by delicate filaments. I do not think all

the forms were alike, and the difference in the method of formation that I have suggested might explain this, and also account for the variations observed in thickness, those forms in which there was a definite periplastic sheath enclosing the separate bodies being the broader.

If I am correct in interpreting all these dissimilar forms as being morphological variations of a single species, *S. eurygyrata*, their occurrence in this patient must, I think, be attributed to the fact that the spirochaetes were multiplying in the urine instead of in the intestinal contents, the unusual nature of the medium determining the production of unusual forms of the organism.

IV. A SPIROCHAETE FOUND IN THE THROAT IN A CASE OF CHRONIC PHARYNGITIS

Some points of interest were raised by a study of the morphology of a spirochaete found in the human throat.

The patient, a native of Accra, a man of about thirty years of age and by employment a cook, applied for treatment at the hospital in January, 1916, complaining of his throat. He stated that his illness had commenced about a year previously with a sore throat, that he had suffered from the same complaint off and on ever since, and that during the last month he had been greatly troubled with a cough, especially at night time. On admission no very definite signs were detected in the chest, but the breath sounds at both apices were harsh, there was slight dullness over the base of the left lung, and a few moist sounds at the base of the right lung. The throat, however, was slightly inflamed and appeared to be in a chronic catarrhal condition, but there was no ulceration. The sputum, which was copious and watery, seemed to come entirely from the throat. In it there were a few red corpuscles, a number of vacuolated polymorphonuclear leucocytes, and epithelial scales. No tubercle bacilli could be found, but fusiform bacilli and very large numbers of spirochaetes were present.

The materials examined were the sputum, which appeared to come entirely from the throat, and the muco-purulent discharge obtained directly from the throat by means of a sterile swab. The

patient's mouth and teeth were first thoroughly cleansed by means of a stiff brush, the mouth and throat were then washed with carbolic lotion (1 in 40), and finally rinsed out with sterilised water. The sputum and swabs were examined immediately after they left the body, and both were found to contain the same organisms in approximately the same numbers.

Under treatment the patient improved considerably, and his general health became much better. The chronic catarrhal condition of the throat, however, proved refractory. When last seen, in August, 1916, his voice was still very husky, his throat was slightly red and covered by a small amount of muco-purulent discharge, and he was still expectorating a considerable quantity of fluid. No spirochaetes could be found in the sputum at this date.

MORPHOLOGY OF THE SPIROCHAETE

The spirochaetes when alive were generally active, and their movements varied considerably, but did not show any characters of specific importance. They stained readily with any of the usual reagents, and were decolourised by Gram's method. Their bodies were flexible and sinuous, somewhat thicker in the middle, and tapering at the ends sometimes to sharp points, sometimes to slightly blunter extremities (fig. IV, 1-48). Some of the spirochaetes were loosely, others closely coiled. The actual number of waves into which their bodies were bent varied greatly, some showing only one or two, others six or more, but the majority had perhaps three or four. The number of waves varied with the length and the thickness of the organism, and also with the nature of its movements. The periplast of some of the spirochaetes was extended laterally into a definite sinuous membrane or crest, or terminally into a delicate appendage. Both these structures were, however, impossible to distinguish in most of the organisms.

Multiplication appeared to occur by both longitudinal and transverse division. Spirochaetes composed of two daughter organisms, linked end to end by a delicate filament, were not uncommon (fig. IV, 46-48); but the Y-forms that suggested longitudinal division were rare (fig. IV, 45). Although epithelial scales were abundant, both in the sputum and in smears taken directly

from the throat, no indications of an intracellular phase were observed. The occurrence of a few very small individuals, measuring only 3μ or 4μ in length (see Table III), suggested, however, that such a phase might occur.

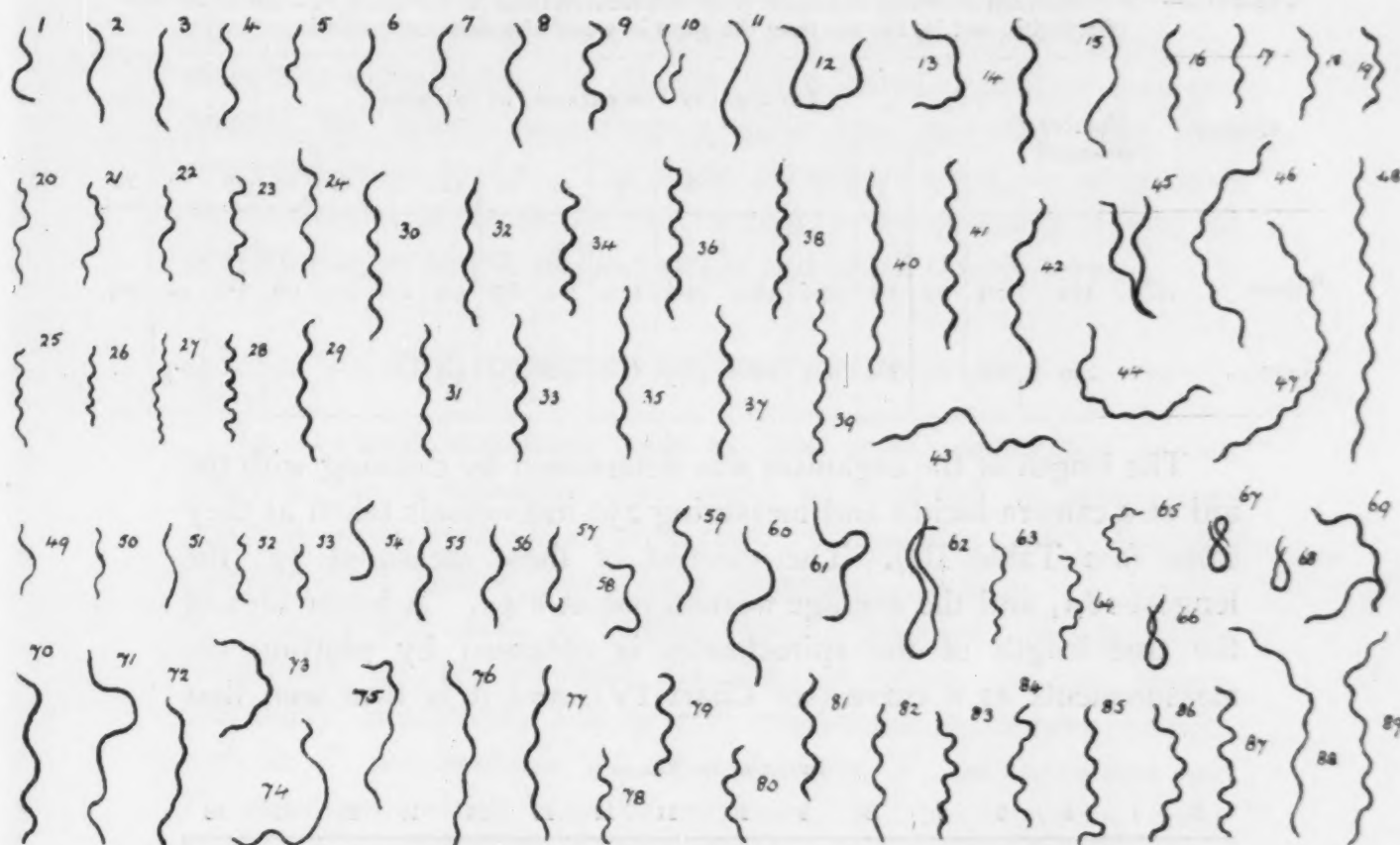


FIG. IV.—1-48: spirochaetes from the throat of a case of chronic pharyngitis; 49-89: spirochaetes from the gums of a case of pyorrhoecia alveolaris. All the figures were drawn with the aid of an Abbé-Zeiss camera lucida at a magnification of 2000 diameters; the thickness of the spirochaetes is, however, only approximately indicated.

The cytoplasm of the majority of the spirochaetes appeared to be homogeneous and non-vacuolated, but a number of them showed a banding of the body by chromatin rodlets, and not a few contained coccoid bodies.

Tangles were occasionally seen in the stained preparations, and also recurved forms.

The spirochaetes varied in breadth according to the stain employed, and on account of their activity could not be measured when alive. Even in the same preparation, however, considerable variations occurred, some individuals being twice as thick as others,

but the breadth did not appear to bear any direct relationship to the length. Accurate measurements were in any case almost impossible owing to the slenderness of the organisms, but the majority probably measured about 0.25μ .

TABLE III.—The distribution according to lengths of the spirochaetes from (a) the throat in a case of chronic pharyngitis, and (b) the pus from the gums in a case of pyorrhoea alveolaris.

Habitat	Number measured	LENGTHS, BY PERCENTAGES, IN MICRONS															
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Throat ...	250	0.4	1.2	7.2	14.0	18.0	19.6	13.2	7.2	6.8	5.2	2.0	2.0	1.6	1.2	...	0.4
Gums ...	200	1.0	4.5	8.5	14.5	18.0	18.5	11.0	9.0	5.5	4.5	3.0	1.0	0.5	...	0.5	...

The length of the organism was determined by drawing with the aid of a camera lucida and measuring 250 individuals taken as they came (see Table III). The shortest of these measured 3μ , the longest 18μ , and the average worked out at 8.4μ . A better idea of the true length of the spirochaetes is obtained by plotting the measurements as a curve (see Chart IV), and it is then seen that

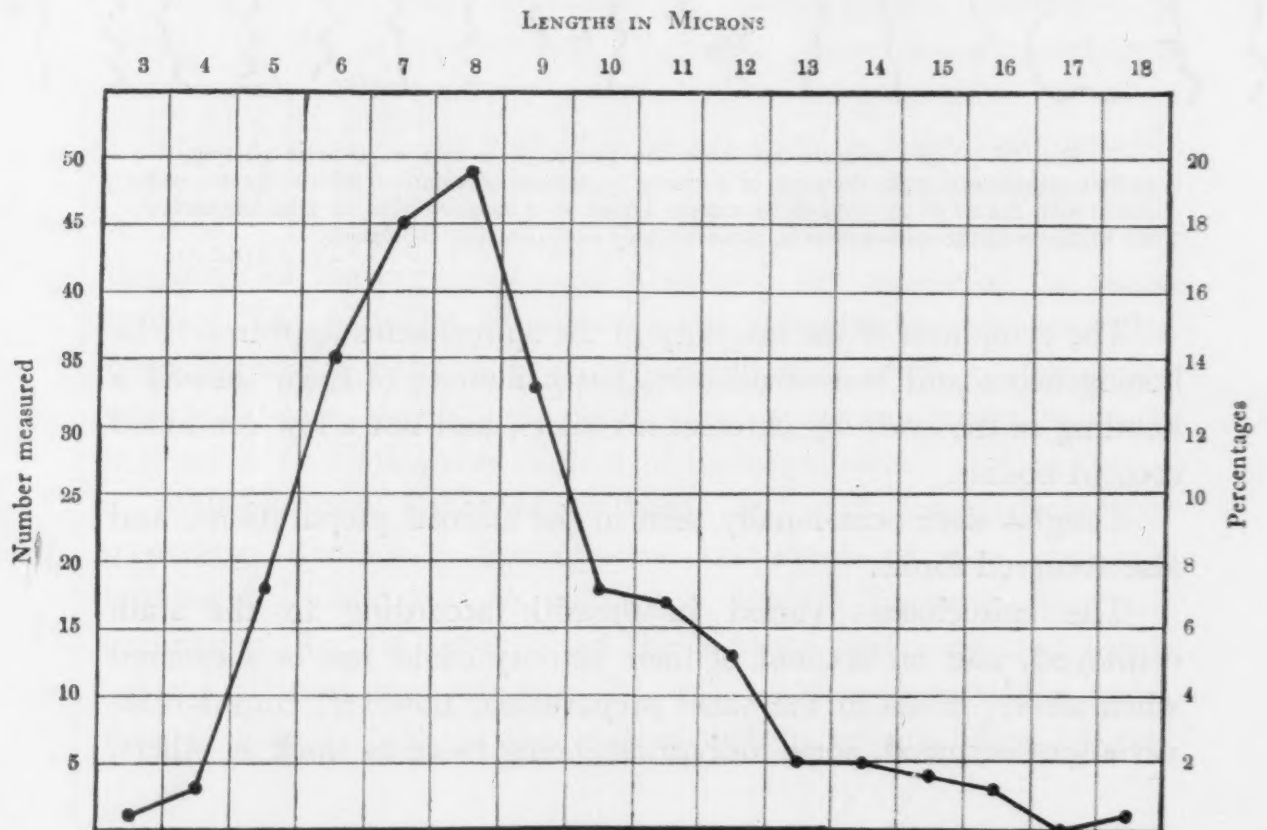


CHART IV.—The distribution according to length of 250 spirochaetes from the throat of a native at Accra.

64.8 per cent. of the organisms measured within 4μ of each other, in length between 6μ and 9μ . This curve is of the form typical of the variations of a single organism, and proves clearly, I believe, that all the spirochaetes found in this patient were of the same species. Both pre-division forms and forms made up of two daughter spirochaetes still attached were included in these measurements, the latter measuring usually 13μ to 16μ , and their components 6μ to 8μ . The slight subsidiary crest, or rather halt in the descent of the curve, at 11μ to 12μ was probably due to the overlapping in length of long single and small double forms.

THE IDENTITY OF THE SPIROCHAETE

In a throat condition such as that from which this patient suffered, the spirochaetes that might be expected to occur most probably are *S. vincenti*, *S. bronchialis*, *S. dentium*, and *S. buccalis*. Certain other species have been described, such as *S. gracilis*, *S. microgyrata*, and *S. media*, which, however, are generally considered to be referable to one or other of the species previously mentioned; and several treponemas have been named, such as *T. intermedium*, *T. macrodentium*, *T. microdentium* and *T. mucosum*, which may be excluded, as the organism under discussion sometimes had a so-called membrane and its coils were variable. For the same reasons *T. pallidum* must be excluded.

Spirochaeta vincenti, which characteristically occurs in the throat, is described as having a flexible, irregularly coiled body, tapering at both ends, and is found associated with fusiform bacilli. In these respects it resembles the organism in the case described above. *S. vincenti* is, however, stated by some authors to be sluggish; Plaut has described a euglenoid type of movement; and others, such as Goadby (1916), consider it to be non-motile. As regards measurements, Bosanquet gives the usual length as 10μ , but states that Mackie records specimens measuring as much as 40μ ; Castellani and Chalmers give 12μ to 25μ ; and Fantham (1915) mentions some measurements ranging from 9μ to 23μ made by him from the drawings published by Thomson and Mühlens, but points out that little is known of the range of morphological variation of this species. Without going into further details, it is evident,

I think, that the active organism, measuring most commonly 6μ to 9μ , that I have described cannot have been *S. vincenti*.

With regard to *S. bronchialis*, a parasite originally described by Castellani, nothing definite can be said, as more recently it has been admitted by this author with Chalmers that the name 'probably includes several varieties of spirochaetes.' From my own observations at Accra (1915), I concluded that the organism found in cases of bronchial spirochaetosis averaged 8μ to 9μ in length, and was indistinguishable from a mouth spirochaete. Fantham has studied the morphology of *S. bronchialis* more recently, and states that its length may range from 5μ to 27μ . He considers that it is distinct from the spirochaetes occurring in the mouth, but his reasons are not very convincing, and the question cannot be regarded as settled.

The two common mouth spirochaetes, *S. dentium* and *S. buccalis*, might be expected to occur in lesions of the throat. These organisms have recently been studied by Fantham (1915). *S. dentium* is said to measure from 4μ to 10μ in length, and from 0.3μ to 0.6μ in breadth, to have a number of well-marked regular coils, and to resemble *T. pallidum* in general appearance, but to be thicker, shorter, and rather less sharply coiled. Mühlens figures chromatin granules in this organism, but Fantham states that it is so small that few structural details can be made out, and that in consequence 'it might be placed by some authorities in the genus *Treponema*.'

S. buccalis is a larger, thicker organism, resembling *S. vincenti*. It is said by some to be active (Mühlens), by others to be sluggish (Goadby); its body is loosely coiled; its extremities are 'somewhat rounded or bluntly acuminate' (Fantham); it has terminal periplastic appendages, and a membrane or crest may be present (Hoffmann). Its length is variously stated as 12μ to 20μ (Hartmann and Mühlens), and 9μ to 22μ (Fantham), and it is said to be 0.5μ to 1μ broad.

The spirochaete that I have described differed from both these species in some particulars, and resembled them in others. It was longer than *S. dentium*, sometimes had a membrane and terminal periplastic appendages, and was not always closely coiled. It was shorter than *S. buccalis*, its ends were not as a rule blunt, and its coils were not always loose.

Intermediate forms which might be considered to connect *S. dentium* and *S. buccalis* are known to occur in the mouth, and to these Dobell has given the name *T. intermedium*. It is possible, however, that *S. dentium* and *S. buccalis* may be morphological variations of a single species. If this were so, the organism would range in length from 4μ to 20μ or 22μ ; would be sometimes loosely, sometimes closely coiled; would have pointed or rather blunter ends; would show in some specimens a membrane, terminal periplastic appendages, chromatin rodlets, and coccoid bodies; would generally be active, but sometimes more sluggish; would occur as tangles occasionally; would show signs of multiplication by both longitudinal division and transverse fission; but would not show intracellular stages. This description corresponds almost exactly with that of the spirochaete I have given above.

As I have already pointed out, I believe that the curve of the measurements of length of the spirochaetes from the throat in the case I have described, proves that the infection was not a mixed one, but was with a single species; and from what has just been stated, this organism appears to combine the characters of *S. dentium* and *S. buccalis*. For this parasite I propose the provisional name *Spirochaeta bucco-pharyngei*, although it is possible that later it may have to be merged with *S. dentium* and *S. buccalis*, in which case the name *S. buccalis* will have priority.

In this connexion it may be of interest to record the fact that an apparently identical spirochaete was found in a severe case of pyorrhoea alveolaris in a native man. In the pus that welled up from the sockets of the teeth on pressing over the gums of this patient, both amoebae and spirochaetes were found. The latter organisms were present in myriads, and, so far as their structure was concerned, resembled exactly the spirochaete from the throat described above (fig. IV, 49-89). They were very active when alive, and showed lashing, undulatory, and helicoid movements. Two hundred individuals, taken as they came, were drawn with the aid of a camera lucida, and measured. The longest of these was 17μ , the shortest 3μ , and the average worked out at 7.97μ (see Table III); the majority, 62 per cent., were within four microns of each other in length between 6μ and 9μ . These measurements and the curve of the distribution of the spirochaetes according to length (see

Chart V) so closely resemble those of the throat spirochaete that it must be concluded they were the same. The spirochaetes in the pus from the gums were presumably mouth spirochaetes, and the nature of their variations according to length support the view I have suggested, namely, that *S. buccalis* and *S. dentium* may really be different forms of the same organism.

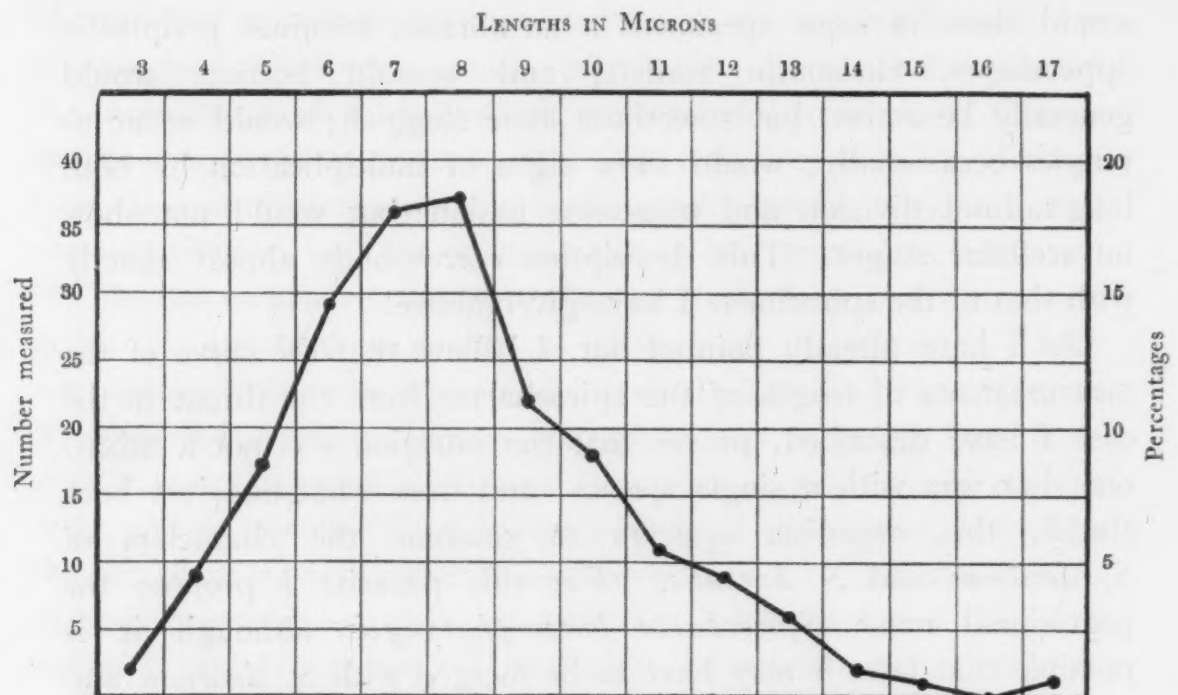


CHART V.—The distribution according to length of 200 spirochaetes from a case of *Pyorrhoea alveolaris* in a native at Accra.

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V. OBSERVATIONS ON THE INTESTINAL SPIROCHAETES OF CERTAIN OF THE LOWER ANIMALS

It is well known that spirochaetes are common in the alimentary canals of animals, but the knowledge regarding them appears to be fragmentary. Until quite recently, even the intestinal spirochaetes found in man had not been thoroughly studied, and with the exception of a few brief references, I am unable to discover in the literature at my disposal any descriptions of the forms found in domestic animals. The indifference hitherto displayed towards these organisms is no doubt due to the fact that most of them appear to be, or are assumed to be, non-pathogenic. They are generally regarded as being saprophytes, and therefore of little account; but it may be worth while considering this attitude a little more closely.

Many of the spirochaetes found on the surfaces of ulcerative lesions are also regarded as being saprophytic, but in some cases there is evidence that the organisms may spread beyond the superficial lesions, and even become blood parasites. Baermann, for example, found *S. refringens* in enlarged glands in a monkey infected with syphilis, and Scherber states that *S. balanitidis* may make its way into the blood-vessels. It is surely not impossible, then, that spirochaetes from the intestinal tract might find their way into the blood stream. In the course of examining blood films from the Accra slaughter-house in 1914, spirochaetes were occasionally found: namely, in four out of 166 cattle, four out of 95 sheep, one out of 94 pigs, and one out of 80 goats. The parasites were of types found to occur in the intestines of these animals, and they were always rare in the films. The method employed in making the blood films did not entirely exclude the possibility of contamination, but it would have been a remarkable feat for spirochaetes to have reached the slides in this manner without other recognisable intestinal matter accompanying them, and it is certainly just as probable that they had found their way into the blood stream through the intestinal wall.

The intestinal spirochaetes are sometimes present in such enormous numbers that one cannot but suspect that they are not

entirely harmless. In man they are often most abundant in cases of diarrhoea, and they are certainly suspected of being directly connected with this condition. Fantham (1916) has described the act of penetration of epithelial cells in human faeces by spirochaetes, an observation which suggests that they may similarly enter the cells lining the intestinal canal.

Without unduly insisting on this point, it may be suggested that intestinal spirochaetes are at any rate a potential danger, and may be capable of pathological activity, either by spreading into the blood-vessels and producing a generalised infection, or by invading the cells of the intestinal walls, or even by mechanical irritation.

During the last few months, in connexion with some work on *S. eurygyrata*, I have had occasion to examine a few domestic animals, rats, etc., for intestinal spirochaetes, and a short account of the organisms found in them may be of interest. I shall, however, confine myself to a consideration of those forms resembling more or less closely *S. eurygyrata*, the type found in the human intestine.

I. MONKEY

A little more than a year ago, I found great numbers of small spirochaetes in smears made from the large intestine and rectum of a monkey (*Cercopithecus petaurista*) that had died of amoebic dysentery at Accra. In the brief account of these organisms that I published (1915), it was pointed out that their morphology closely resembled that of *Spirochaeta eurygyrata*, and a few individuals were figured in support of this statement.

I have recently re-examined some of the specimens taken from this monkey, and I have been unable to discover any points of distinction between the spirochaetes found in them and those so common in human faeces (fig. V, 1-24). All the organisms in the monkey were of the loosely coiled type, but, as I have pointed out elsewhere (1916), in many human stools, also, only this type is found.

The apparent thickness of this organism, as of all the others of this type, varies according to the stain employed and the method of fixation used. In my earlier account, from dried smears stained by Leishman's method, the spirochaetes were described as being extremely slender, but after re-staining with gentian violet they appeared to be rather thicker, about 0.2μ .

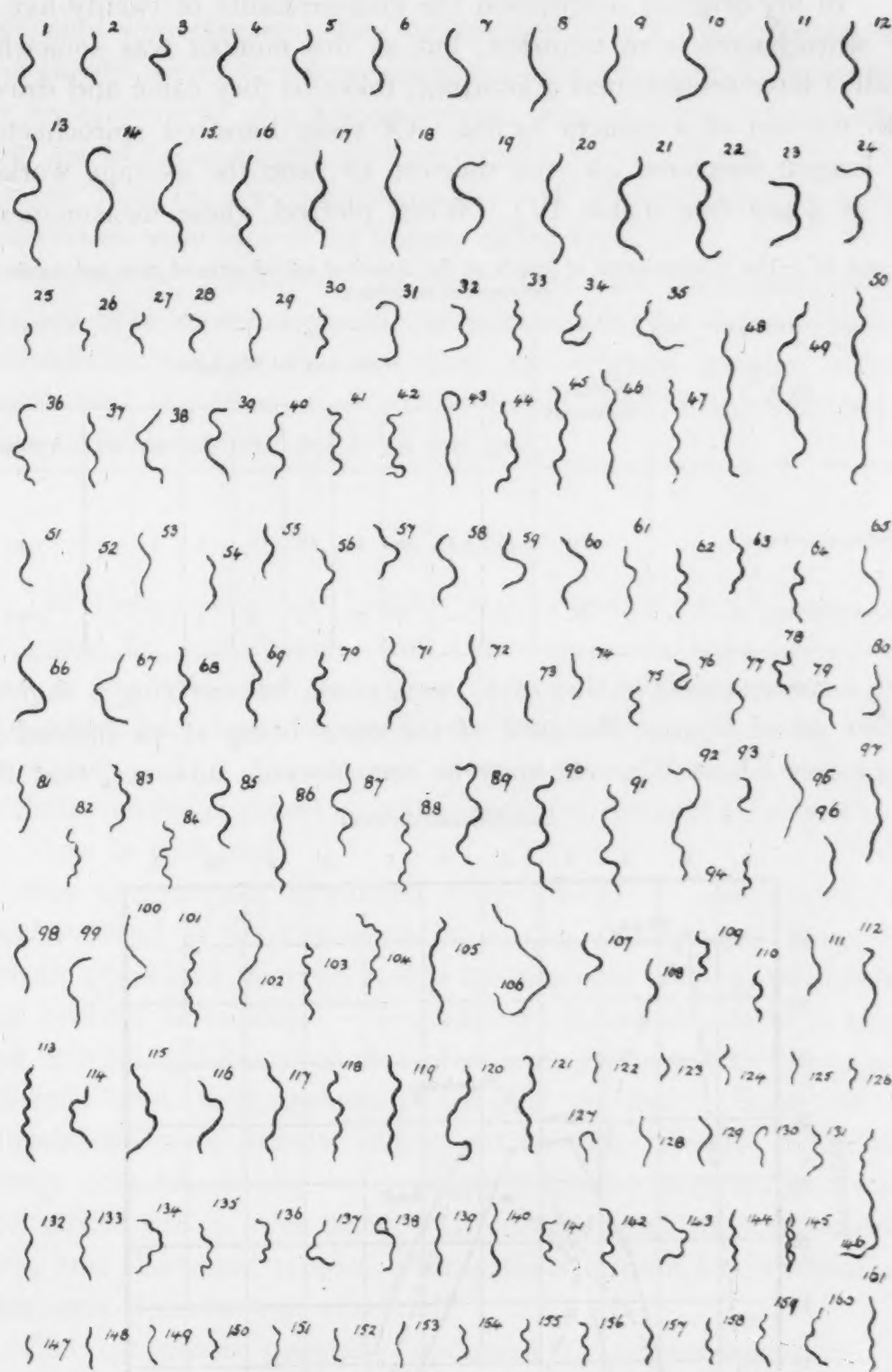


FIG. V.—1-161: spirochaetes from the faeces of various animals, namely:—1-24, a monkey; 25-50, rats; 51-72, sheep; 73-92, cattle; 93-106, goats; 107-121, pigs; 122-146, dogs; and 147-161, cats. All the drawings were made with the aid of an Abbe-Zeiss camera lucida. The magnification is $\times 2000$. The thickness of the organisms is, however, only approximately correct.

In my original description the measurements of twenty-five of the spirochaetes were recorded, but as this number was somewhat small, I have re-measured a hundred, taken as they came and drawn with the aid of a camera lucida. Of these hundred spirochaetes, the longest measured 9μ , the shortest 3μ , and the average worked out at 5.42μ (see Table IV). When plotted, these measurements

TABLE IV.—The measurements of length of the intestinal spirochaetes of man and monkey obtained at autopsies.

Host	Number measured	LENGTHS IN MICRONS									
		2	3	4	5	6	7	8	9	10	Average
Monkey, at autopsy	100	...	3	20	32	26	16	2	1	...	5.42
Man, at autopsy	25	3	10	5	4	3	5.76

form a curve similar to that of *S. eurygyrata*, but covering a slightly higher set of figures, the crest of the curve being at 5μ instead of at 4μ (see Chart VI). It must be remembered, however, that the

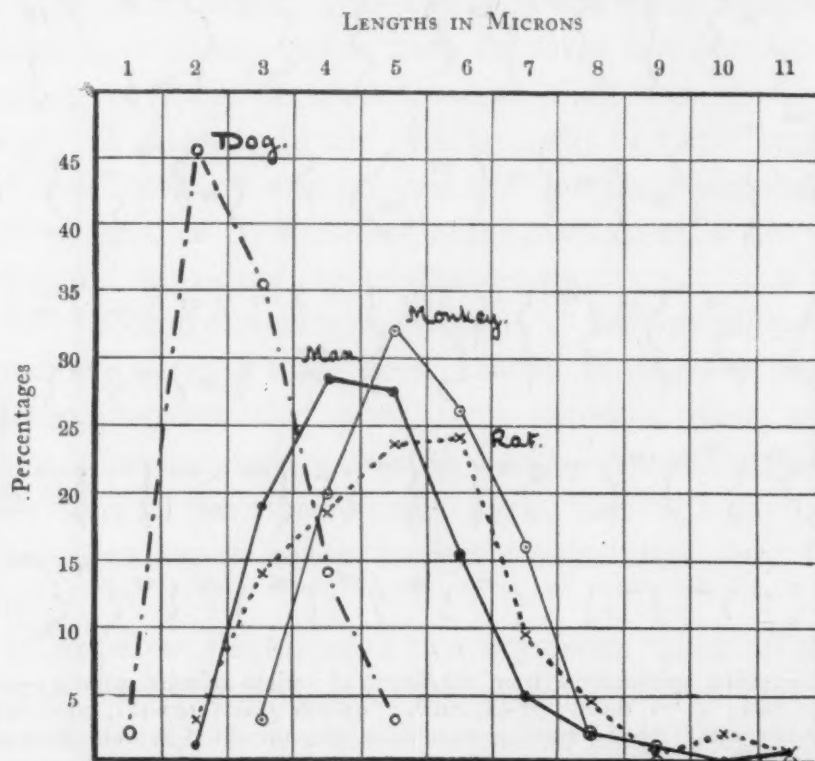


CHART VI.—The distribution, by percentages, of the lengths of the intestinal spirochaetes of man (●—●—), monkey (○—○—), rats (× — × —), and dogs (○—○—).

specimens were obtained from the monkey after death. Twenty-five spirochaetes were, therefore, measured in a preparation made from the rectal contents of a human subject at an autopsy, these ranged from 4μ to 8μ in length, and averaged 5.7μ (see Table IV). The greatest number, ten, measured 5μ . All the spirochaetes in this preparation were also of the loosely coiled type.

It may be concluded, therefore, that this spirochaete of the monkey was morphologically identical with the human species, *Spirochaeta eurygyrata*, and that the slightly greater average length and the absence of closely coiled individuals was due to changes following the death of the host.

2. RATS

The faeces of a few healthy rats, *Epimys rattus*, have been examined for spirochaetes, and these organisms have been found both in the wild and the albino varieties of these animals. The albino rats had been imported into West Africa about two years previously. The spirochaetes were numerous in some of the animals, scanty in others, but the species of parasite appeared to be the same in each case.

The spirochaetes resembled very closely *S. eurygyrata*, the species found in the human intestine (fig. V, 25-50). They were slender organisms, actively motile by means of lashing, undulatory, and helicoid movements. They varied considerably both in length and breadth and in the number of coils they possessed. Their ends tapered, and were generally sharply pointed. Some of the spirochaetes were loosely coiled, others more closely; some were merely bent like a bow, some showed two or three distinct waves and others five or even more. Recurved forms were occasionally seen, and also small tangles. Indications of both longitudinal and transverse division were observed.

The cytoplasm generally appeared to be homogeneous; but some specimens were banded by rodlets at short intervals along their bodies, and coccoid bodies had formed in others. A distinct membrane was never seen, but the appearance of some of the organisms suggested that it might be present.

The spirochaetes varied a good deal in thickness, but did not

measure more than 0.25μ at their broadest part. The length of the organisms varied from 2μ to 11μ , and averaged 5.27μ in the two hundred individuals measured.

From this brief description it will be concluded that the spirochaete could not be distinguished from *S. eurygyrata* by morphological characters, and as Fantham (1916) has recently published an admirable account of the latter organism, it will be unnecessary to enter into greater detail. The general characters of the spirochaete are, however, indicated in the rough drawings (fig. V, 25-50), and in Table V some details of the measurements of

TABLE V.—The measurements of length of the spirochaetes found in the intestines of man, rats, and dogs at Accra.

Host	Number measured	LENGTHS IN MICRONS											Average
		1	2	3	4	5	6	7	8	9	10	11	
Man	200	...	2	38	57	55	31	10	4	2	...	1	4.69
Rat	200	...	6	28	37	47	48	19	9	1	4	1	5.27
Dog	200	4	91	71	28	6	2.70

length are given for comparison with those of the human species. It should be mentioned, however, that I did not observe the spirochaetes penetrating shed epithelial cells and forming coccoid bodies in them as Fantham did in the case of *S. eurygyrata*.

Two hundred spirochaetes from the rats' faeces, taken as they came, were drawn with the aid of a camera lucida and measured (see Table V). They varied in length from 2μ to 11μ , but the longer individuals were rare, and were often obviously dividing forms composed of two daughter spirochaetes joined end to end by a delicate filament (fig. V, 48-50). The single organism, measuring 11μ , appeared to be composed of three parts, measuring 4μ , 4μ , and 3μ , respectively. A much better idea of the length of the spirochaete is obtained by distributing the measurements and plotting them as a curve (see Chart VI); and it is then seen that 80 per cent. of them were within four microns of each other in length from 3μ to 6μ .

On comparing the curves formed by distributing, according to length, two hundred spirochaetes from the faeces of rats and a

similar number from the human stools, they are found to be much alike. In the case of the rat spirochaete the crest of the curve is at 5μ to 6μ , in the case of the human parasite at 4μ to 5μ ; but in both curves the great majority of the organisms measured from 3μ to 6μ —80 per cent. of the former, and 90·5 per cent. of the latter. The range of variation in length was also practically identical.

The spirochaete found in the rats' intestine resembled *S. eurygyrata*, Werner emend. Fantham, so closely both in structure and detailed measurements that it would be impossible to distinguish the two organisms by morphological characters, and it is at any rate possible, although one hesitates to make the assertion without further evidence, that they may be the same species.

3. SHEEP, CATTLE, GOATS, AND PIGS

A few sheep, cattle, goats, and pigs have also been examined, and in all of these spirochaetes of the type under consideration have been found in the alimentary canal and in the faeces. The parasites appeared to be identical in all these animals, and they were indistinguishable, both when alive and after fixation and staining, from the organisms found in rats and man. The brief description of the rat spirochaetes given above may be read word for word as applying to these organisms, and the rough drawings (fig. V, 51-121) will serve to illustrate the morphological similarity.

Twenty-five spirochaetes, taken as they came from each species of animal, were drawn with the aid of a camera lucida and measured (see Table VI). The average length was $4\cdot7\mu$ in sheep,

TABLE VI.—The measurements of length of the intestinal spirochaetes of various animals examined at Accra.

Host	Number measured	LENGTHS IN MICRONS										Average
		1	2	3	4	5	6	7	8	9	10	
Sheep	25	3	7	11	3	1	4·7
Cattle	25	8	7	2	3	2	3	4·7
Goat	25	3	3	8	5	4	1	...	1	5·5
Pig	25	3	6	6	6	2	...	2	...	5·2
Cat	25	1	11	9	4	2·4

4.7 μ in cattle, 5.5 μ in goats, and 5.2 μ in pigs. Considering the small number of measurements made in each case, the lengths correspond fairly closely with each other and with those of the rat spirochaetes and *S. eurygyrata*.

A series of smears from various parts of the alimentary tract of one sheep were examined, and in them spirochaetes of this type were found in the specimens from the stomach, as well as in those from the intestine.

It may be mentioned that the majority of the spirochaetes found in blood films from the Accra slaughter-house (1914), to which reference has been made, were of the same type. The measurements and illustrations given in the Report of the Accra Laboratory for the year 1914 clearly show this. It is possible, therefore, that under certain conditions the organisms may find their way from the intestine into the blood stream, in which case they would have to be distinguished from *S. theileri*, the parasite found by Theiler in cattle suffering from a disease characterised by fever, diarrhoea, and splenic enlargement, which is a much longer organism, measuring 20 μ to 30 μ in length.

4. DOGS

In the faeces of an apparently healthy rough-haired terrier of European extraction an exceedingly heavy infection with minute spirochaetes was found (fig. V, 122-146). The dog had lived for a long time in West Africa and was thoroughly acclimatised, and at the time of examination did not appear to be suffering from any pathological condition. The same organism has also been found in two native dogs; the infection in one being very heavy and in the other slight. A third native dog examined did not appear to harbour any spirochaetes.

The living spirochaetes were active, but their movements did not show any characteristics of specific importance. They were very small, and in consequence structural details were difficult to observe. When fixed and stained the spirochaetes were seen to be delicate organisms of varying size, with flexible bodies bent into an inconstant number of waves. Their ends were tapering and generally sharply pointed, but the shorter and thicker individuals had somewhat blunter extremities. The body was as a rule loosely,

but sometimes closely coiled, and the number of separate waves varied greatly. Some specimens were bent into a simple bow, others showed four or even more closely set waves, but the majority had one or two relatively loose coils. A few tangles were found in the films.

The cytoplasm of most of the spirochaetes appeared to be homogeneous, but a few were definitely banded by chromatinic rodlets. Coccoid bodies were frequently visible. The membrane, if it was present, was indistinguishable, but the organisms were so small that this structure could scarcely have been visible under any circumstances. Spirochaetes in process of transverse fission were frequently seen, and a few multiple forms were present. Other organisms were observed which appeared to be undergoing longitudinal division.

Although the spirochaetes were abundant in all parts of the faeces, they were most numerous in the mucus contained in it. The cells in the excrement did not appear to have been invaded by the parasites, and the small number of spirochaetes that looked as if they were within them may really have been lying on their surfaces. No definite evidence of an intracellular phase could be discovered.

The spirochaetes were slender organisms, but varied considerably in thickness. The smaller individuals were relatively the stouter. Accurate measurements were, of course, impossible, but even the thicker ones were less than 0.25μ broad.

The length of the parasite was determined by drawing two hundred individuals, taken as they came, with the aid of a camera lucida, and measuring them. The shortest length was 1μ , the longest 5μ , and the average worked out at 2.7μ (see Table V). The great majority of the spirochaetes, 81 per cent., measured 2μ to 3μ (see Chart VI). Elsewhere in the films a very few longer organisms were found, some of which reached 9μ (fig. V, 146). These were apparently multiple forms. The infected animals were examined on several occasions at intervals of a week or ten days without observing any morphological changes suggesting that these small spirochaetes were a stage in the development of a larger organism. In general character the spirochaete approximated to the *S. eurygyrata* type, but a comparison of the curves made by plotting the measurements of length of the two organisms shows clearly that they were not

identical. The spirochaete found in the dogs was a much smaller organism with a more restricted range of variation.

Various spirochaetes have previously been described as occurring in the alimentary canal of dogs by Oppenheimer, Bizzozero, Salomon, and Rigaud. I have not been able to consult the works of these authors in the originals, but, according to Bosanquet, the organisms discovered by Bizzozero lay within vacuoles in the epithelial cells, showed three to seven coils, and measured 3μ to 8μ in length, and may have been the same as those found by Oppenheimer. Salomon's spirilla were longer and had terminal flagella, and the organisms found by Rigaud resembled *T. pallidum*. The spirochaete I have described above resembled none of these organisms. Balfour (1906) discovered spirochaetes in the stomach and intestines of dogs dying of experimental trypanosomiasis in the Sudan, and eight specimens from a gastric ulcer are figured in a beautiful coloured plate (XIV) in the Second Report of the Wellcome Research Laboratories, Khartoum. They appear to have been thicker and much more closely and regularly coiled organisms than those I have described; and, judging from the individuals figured, longer (6μ to over 10μ). I have found what I believe to be this species of (?) spirochaete in the stomachs of dogs and a cat (see p. 341), and if I am correct in the identification, it is an entirely different parasite.

For descriptive purposes it will be convenient to name the spirochaete found in the dogs at Accra, and I, therefore, propose that it should be called *Spirochaeta canis*.

5. CATS

The faeces of four cats have been examined, and in three a few, but only a few small spirochaetes were found. The organisms appeared to be identical with those found in dogs (*S. canis* n. sp.), a description of which has already been given (fig. V, 147-161).

Twenty-five individuals, taken as they came and drawn with the aid of a camera lucida, measured from 1μ to 4μ , but averaged 2.4μ in length (see Table VI). Twenty of the twenty-five measured 2μ to 3μ . The general form of the spirochaete is shown in fig. V, and it will be seen that it exactly resembles that of the organism found in the dog.

These three cats were obtained from the house of a native.

In the fourth cat two distinct spiral organisms were found, the one in the stomach and the other in the large intestine and rectum. This animal was examined only after death, its body having been sent to the laboratory for dissection by its European owner.

The organism found in the stomach was not of the *Spirochaeta eurygyrata* type, so that it does not properly concern us here. It was, however, a relatively thick organism, so closely coiled that it resembled a screw with a narrow thread (fig. VI, 1 and 2). The ends

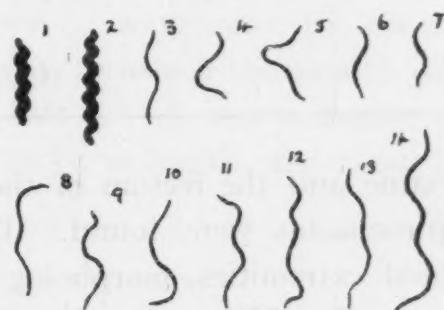


FIG. VI.—1 and 2: spiral organisms from the stomach; and 3-14: spirochaetes resembling *S. eurygyrata* from the large intestine and rectum of a cat examined after death. All the figures were drawn with the aid of an Abbé-Zeiss camera lucida at a magnification of 2000 diameters.

of the parasite were really blunt, but as they were parts of incomplete coils they stood out slightly, giving the appearance of pointed extremities to the whole organism. The actual breadth of the parasite was about 0.4μ or 0.5μ , but the body was coiled so closely and so evenly that the apparent breadth was really the diameter of the spiral, and this was about 0.8μ . The diameter of the spiral was constant from end to end of the organism, and did not vary in different individuals. The length of the body varied considerably, but could not be measured owing to the closeness of its coils; but some idea of the range may be gathered from measurements of the axial length. With the aid of a camera lucida, the axes of twenty-five individuals, taken as they came, were drawn. They ranged in length from 3μ to 8μ , and the average was 5.48μ (see Table VII). In the same individuals the number of coils varied from 3 to 10, and averaged 5.9. The coils appeared to be fixed. This organism was, I think, the same as that found by Balfour in the stomach of a dog, and figured in the Second Report of the Wellcome

Research Laboratories, Khartoum, and should probably be referred to the genus *Treponema*.

TABLE VII.—The lengths in microns and the number of coils of twenty-five individuals of a spiral organism found in the stomach of a cat.

Morphological feature	LENGTHS IN MICRONS OR THE NUMBER OF COILS							
	3	4	5	6	7	8	9	10
Lengths in microns	1	4	7	7	5	1
Coils	1	6	6	5	3	1	1	2

In the large intestine and the rectum of the same cat a great number of small spirochaetes were found. These were delicate organisms with pointed extremities, morphologically indistinguishable from *S. eurygyrata* (fig. VI, 3-14). It will be unnecessary to give a detailed description of these spirochaetes, as already the type has been considered more than once in this article, and the description already given may be re-applied here. They were all loosely coiled in this cat, but a similar absence of closely coiled specimens was observed in *S. eurygyrata* obtained from the human subject after death, and in the case of the monkey already referred to. A hundred individuals, taken as they came, were drawn with the aid of a camera lucida, and measured. They ranged from 3μ to 11μ , and averaged 5.78μ in length. When distributed according to their lengths it was found that 86 per cent. of them measured within four microns of each other in length between 4μ and 7μ (see Table VIII). These measurements of length are almost identical

TABLE VIII.—The measurements of length of a spirochaete found in the large intestine and rectum of a cat.

Host and Habitat	Number measured	LENGTHS IN MICRONS									
		3	4	5	6	7	8	9	10	11	
Cat, large intestine and rectum	100	3	13	33	23	17	7	2	1	1	

with those of the spirochaete found in the large intestine of a monkey which had died of dysentery, and it must be concluded that this organism also was morphologically identical with *S. eurygyrata*.

CONCLUSIONS

1. Spirochaetes of the *S. eurygyrata* type have been found in the faeces of certain of the lower animals examined at Accra.
2. The first type was found in a monkey, a cat, rats, sheep, cattle, goats, and pigs, and appeared to be morphologically indistinguishable from *S. eurygyrata*, the species found in man.
3. The second type, for which the name *S. canis* is proposed, was found in dogs and cats. This was a smaller organism, measuring most commonly 2μ to 3μ in length, and about 0.2μ in breadth.

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STUDIES IN BLACKWATER FEVER*

V.—ON THE IMPORTANCE OF FURNISHING POPULATION STATISTICS IN CONNEXION WITH CASES OF BLACKWATER FEVER

BY

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In the various reports which I have read during the last few years on blackwater fever, mainly in British Colonies, statements are met with in regard to the sex, age and race distribution, and other factors which have a bearing on the aetiology of the disease, which from the fact that they are unaccompanied by particular information, more especially population figures, are not capable of satisfactory interpretation. Examples of these incomplete statements follow. I think it will be obvious from the examples I give that owing to their incompleteness they are valueless, but that the additional information required—if it could be given, and I believe it could—would render them valuable, and so help to solve the blackwater problem.

(1) *Sex distribution of Blackwater cases*

- (a) 'Of the total cases two were females and fifty-six males.'
- (b) 'Only one of the nineteen (European) cases was a female.'
- (c) 'Of the seven cases recorded all were males. Six European and one Indian.'

No conclusion is possible, as the relative numbers of males and females is not known.

* Part I: *Annals of Trop. Med. & Parasitol.*, 1913, p. 479. Part II: *Ibid.*, 1914, p. 639.
Part III: *Ibid.*, 1915, p. 201. Part IV: *Ibid.*, 1915, p. 429.

(2) *Age distribution of Blackwater cases*

(a) 'There were three children ages 4, 7, and 13.'

(b) 'Most of the patients (nine) were between 30 and 40 years.'

No conclusion is possible, as the numbers of people at various age periods are not given. It is stated, however, in one paragraph 'That the age period 30 to 40 years includes a larger number of Europeans than any other, although the age period 20 to 30 years runs it close,' but no data are given in support of this statement.

(c) 'A table is given of the age distribution of twenty-one cases of blackwater, but this is quite a different matter and does not really help us.

What we want to know is the age distribution of the *population*, then, knowing the ages of a number of cases, we can calculate the distribution for each age group.

(3) *Racial distribution of Blackwater*

(a) 'Of the seven cases recorded all were males, six European and one Indian.'

(b) 'Sixteen Europeans and one Syrian were attacked.'

(c) 'Nineteen cases occurred amongst Europeans, one in a West Indian Negro.'

No conclusion is possible in absence of relative populations.

(4) *Effect of length of residence on liability to Blackwater*

(a) 'Thirteen of the cases occurred in those who had served five years or less. This is considerably more than half the total.'

(b) 'Length of residence in Africa varies from six weeks to twenty-two years. In four cases it was over ten years.'

No conclusion is possible as to the effect of residence, as we do not know the proportions of those who had served five years or less to those who had served five years or more, and so for the second example.

(5) *Does one attack of Blackwater predispose to another?*

'It is noteworthy that four had previously had one attack of blackwater. In the remaining three it was the first attack.'

Whether one attack predisposes to a second can only be shown if we know the respective numbers of those who have previously had one attack and those who have previously had none.

The information almost always given under this heading, as in this example, is the number of *cases* in each category. We must refer the number of cases to a basis of, say, 100 *people* in each category.

No conclusion is then possible without population data.

(6) *Geographical distribution of Blackwater in Africa*

It would undoubtedly be of value to know if blackwater is more prevalent in one colony than another. In various reports we have the following data:—

	No. of cases	POPULATION		
		Europeans	Asiatic	Miscellaneous
Gold Coast	19	?	?	—
Nyasaland	7	?	?	—
Gambia	0	?	?	—
Uganda	58	823	3,110	—
N. Nigeria	17	?	?	—
S. Nigeria	21	1,065	?	—
S. Leone	10	?	To be given in future	—
E. A. Protectorate ...	15	1,075	?	—

If the populations were given, even though decennial, I think it possible that after a series of years information of value might be deduced. But if the *official population* figures only were given, although the figures would be small, even more accurate conclusions could be drawn, and, of course, the blackwater cases would then have to be classified as (1) Official, (2) Non-official.

(7) *Seasonal distribution and relation to Rainfall or Malaria*

(a)

				Table of monthly distribution	Rainfall chart	Malaria statistics
Gold Coast	Not given	Not given	Not given
Nyasaland	Not given	Not given	Not given
Gambia	No cases	Not given	Not given
Uganda	Given	Given	Given
N. Nigeria	Given	Not given	Not given
S. Nigeria	Not given	Given	Not given
S. Leone	Not given	Not given	Not given
E. A. Protectorate	Given	Not given	Not given

(b) 'As regards seasonal prevalence, three of these cases (7) occurred during the rains.'

This statement by itself is of practically no value, but if in each colony a table of monthly prevalence were given, after a series of years I think reliable conclusions could be drawn, although the number of cases in each month may not have been corrected for variations in population. At the same time the rainfall should be given, and any figures for malaria that are available. There is a want of uniformity here in the manner in which the data are presented. Nor is it possible always where a seasonal table is *not* given to construct one oneself, for in some of the reports all the cases are not recorded, but merely a summary given.

(8) *Annual variations in case rate*

'It is difficult to account for the marked increase in the number of cases which occurred during 1913.'

It is doubtful from the data supplied if there is 'a marked increase,' but if there were it might simply be due to an increase in the population, but no information is given on this point.

(9) *Annual variations in death rate*

The form in which these are stated is not always correct. For example, with seventeen cases and eight deaths it is, so far as it

goes, correct to say that the mortality is 47·05 per cent., but in the next 100 it might be 39 or 55, or even 15 to 78, and so comparisons made as to the fall or increase in the *annual* death rate will be incorrect unless this range of variation is allowed for.

(10) *Regional distribution*

(a) 'In forty-two cases the disease appears to have been contracted in a town or station, and in fourteen in the outlying districts, etc.'

(b) 'It will be seen from the chart that thirty-three cases, or more than half, are returned from K. Although this is the largest centre of native and Asiatic population, the proportion is still excessive.'

As the Asiatic and European populations of K. and the other districts mentioned in the chart are not given, it does not appear how this conclusion is reached.

(c) 'With reference to locality, five of the cases occurred in the highlands.'

No conclusion as to the regional distribution of the disease is possible in absence of population records of regions compared.

(11) *Occupation*

(a) 'Four of the patients were by occupation planters, one a Portuguese officer, etc.'

As we do not know the relative populations of planters, officers, etc., no information of real value is given.

(12) *Cases amongst Officials and Non-officials*

Gold Coast	No statement
Nyasaland	Given
Gambia	No statement
Uganda	No statement
N. Nigeria	No statement
S. Nigeria	Given
S. Leone	No statement
E. A. Protectorate	No statement

There is here no uniformity in the method of presenting the data.

I here append a form that might be used in the compilation of official data. In the case of officials, all particulars are, I believe, available, although the number of cases among these will naturally be only a part of the whole; yet less numerous, accurate data from each locality, which in the course of some years will suffice for the drawing of conclusions, will be of value, whereas more numerous, incomplete or inaccurate data will never be of any.

The exact form in which these tables are here drawn up is not essential, but whatever procedure is adopted it should be the same year after year, and the same in each colony or district.

STATISTICS OF OFFICIALS.*

1. Colony

2.	Month				No. of officials	B.W. cases	B.W. deaths
January	—	—	—
February	—	—	—
March	—	—	—
April	—	—	—
May	—	—	—
June	—	—	—
July	—	—	—
August	—	—	—
September	—	—	—
October	—	—	—
November	—	—	—
December	—	—	—
Totals	—	—	—

* Statistics of Official population and other data should be given, even if no cases of blackwater occur during the year.

3. African service	No. of officials	B.W. cases	B.W. deaths
Less than $\frac{1}{2}$ year	—	—	—
More than $\frac{1}{2}$ year	—	—	—
More than 1 year	—	—	—
More than 2 years	—	—	—
More than 3 years	—	—	—
More than 4 years	—	—	—
More than 5 years	—	—	—
6-10 years	—	—	—
11-20 years	—	—	—
21 years and upwards	—	—	—
Totals	—	—	—

4. Age period	No. of officials	B.W. cases	B.W. deaths
0-20	—	—	—
21-25	—	—	—
26-30	—	—	—
31-35	—	—	—
36-40	—	—	—
41-45	—	—	—
46-50	—	—	—
51 upwards	—	—	—
Totals	—	—	—

5. Previous attacks of blackwater	No. of officials	B.W. cases	B.W. deaths
0	—	—	—
1	30*	3*	—
2	—	—	—
3	—	—	—
3 or more	—	—	—
Totals	30	3	—

* These three cases are accordingly second attacks, and have occurred among 30 officials, each of whom has previously had one attack.

6. Attacks of malaria during previous six months	No. of officials	B.W. cases	B.W. deaths
0	—	—	—
1-5	—	—	—
6-10	—	—	—
11-20	—	—	—
21-25	—	—	—
25-30	—	—	—
'many'	—	—	—
Totals	—	—	—

7. Quinine Prophylaxis	No. of officials	B.W. cases	B.W. deaths
None	—	—	—
Irregular (grs. — per month) ...	—	—	—
Regular (grs. — per day) ...	—	—	—
Totals	—	—	—

8. Resident in Station*	No. of officials	B.W. cases	B.W. deaths
A.	—	—	—
B.	—	—	—
C.	—	—	—
D.	—	—	—
Totals	—	—	—

* The places selected should be the same every year.

9. Sex	No. of officials	B.W. cases	B.W. deaths
Males	—	—	—
Females*	—	—	—
Totals	—	—	—

* Wives of officials.

STATISTICS OF NON-OFFICIALS

1. Colony

2. Nationality	Population	B.W. cases	B.W. deaths
Europeans	—	—	—
' Syrian '	—	—	—
E. Indian	—	—	—
W. Indian	—	—	—
Miscellaneous	—	—	—
Totals	—	—	—

3.	Month					B.W. cases	B.W. deaths
January	—	—
February	—	—
March	—	—
April	—	—
May	—	—
June	—	—
July	—	—
August	—	—
September	—	—
October	—	—
November	—	—
December	—	—
Totals	—	—

4.	Month					Rainfall	Cases of malaria
January	—	—
February	—	—
March	—	—
April	—	—
May	—	—
June	—	—
July	—	—
August	—	—
September	—	—
October	—	—
November	—	—
December	—	—

5.	Station	Population*	B.W. cases	B.W. deaths
	A.	—	—	—
	B.	—	—	—
	C.	—	—	—
	D.	—	—	—
	Totals	—	—	—

* For separate nationalities, if available, *not* including the native (African) population.

6.	Age period	Population	B.W. cases	B.W. deaths
	0-20	—	—	—
	21-25	—	—	—
	25-30	—	—	—
	31-35	—	—	—
	35-40	—	—	—
	41-45	—	—	—
	46-50	—	—	—
	51 upwards	—	—	—
	Totals	—	—	—

7.	Sex	Population	B.W. cases	B.W. deaths
	Males	—	—	—
	Females	—	—	—
	Totals	—	—	—

Data as to non-officials are it appears not easily available, if at all, but the ideal to be aimed at is to obtain these as well as the official ones. There are many disputed questions concerning black-water fever which should have been settled once for all years ago. It is high time that action should be taken to obtain this information, and an energetic attempt made to get at all the facts about the disease.

REPORT ON THE X-RAY EXAMINATION OF DYSENTERY AND OTHER CASES

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Glasson (1915), in a paper on the 'Localization of Dysenteric Ulcers by X-rays,' concludes as follows:—

'In acute cases I do not think radiography will be of much assistance, but in chronic cases it will be of great assistance, both to the physician and surgeon, as it will give a clear picture of areas where the ulcerative patches are situated.

'Before a radiogram is taken, the patient should be given bismuth subnitrate in 60 gr. doses every two hours during the day for at least six days. On the sixth day a radiogram should be taken, then the bismuth should be discontinued and liquid paraffin given in 2 dr. doses every two hours for twenty-four hours. Then a second radiogram should be taken, and this second one will show where any ulcerative patches may be situated.'

The technique followed corresponded exactly to that laid down by Glasson in the paper quoted above.

The cases under observation were three cases of chronic dysentery, one of paratyphoid, and one of malaria.

CASE I. Dysentery. Pioneer K—.

Plate 1. 6th day. Ascending colon, transverse colon, descending colon to level of iliac crest full of bismuth food. All the shadows normal.

Plate 2. 24 hours later after paraffin. Ascending colon emptying, transverse colon full, descending colon full to level of iliac crest. All the shadows exactly as are frequently seen in normal bowel.

Plate 3. 24 hours later—Nothing in the bowel anywhere to indicate bismuth.

CASE 2. Dysentery. Private U——.

Plate 1. 6th day. Ascending colon, transverse colon, both full of the food. Nothing abnormal.

Plate 2. 24 hours later after paraffin. Traces of the food in the ascending colon, the transverse colon, and the descending colon. (There was nothing abnormal in shadows of this kind, and they corresponded in appearance almost exactly with the shadows seen on Plate 3 of Case 4—the control case.)

Plate 3. 24 hours later. A very indefinite trace of food in the hepatic flexure. None elsewhere. The trace in the flexure differs in no way from similar traces frequently seen in the examination of cases not suffering from dysentery.

CASE 3. Dysentery. Sergeant R——.

Plate 1. 6th day. Ascending colon, transverse colon, both full. Nothing abnormal.

Plate 2. 24 hours later after paraffin. Hepatic flexure, transverse colon, descending colon to sigmoid, all full, all normal in appearance.

Plate 3. 24 hours later.—Not a trace of the food anywhere.

CASE 4. Malaria—Control Case. Seaman S——.

Plate 1. 6th day. Ascending colon, transverse colon, and descending colon, all full, all normal in appearance.

Plate 2. 24 hours later after paraffin. Practically no change.

Plate 3. 24 hours later. Traces in the ascending colon, in the transverse colon, and in the first part of the descending colon. (Compare Plate 2 of Case 2.)

CASE 5. Paratyphoid. Private W——.

Plate 1. 6th day. Ascending colon, transverse colon, and descending colon all full and normal.

Plate 2. 24 hours later after paraffin. Food in transverse colon, in descending colon, and in rectum; also traces at the hepatic flexure appearing exactly the same as in Plate 3, Case 2.

Plate 3. 24 hours later. All the food has passed.

The points from the X-ray point of view are:—

1. A study of the illustrations of the original paper shows nothing which might not equally well be seen on plates taken, under similar circumstances, in persons not suffering from dysentery.

This is fully borne out by the plates taken of the series of cases we observed.

2. The shadows arrowed on the illustrations in the paper do not show any difference from shadows which are frequently seen in any X-ray department when bismuth or barium meals are being watched through a bowel.

3. It is a well-known X-ray fact that when kidney cases are to be examined, special care has to be taken when the patient has been taking bismuth in medicinal doses. Even when purgatives are given, traces of bismuth frequently remain here and there in the bowel.

4. Still more important is another fact—disclosed by X-rays. In ulcer of the stomach, bismuth does not adhere to the raw surface of the ulcer, and the X-ray diagnosis of gastric or duodenal ulcer cannot be made from this point of view. The question immediately suggests itself, 'Why, when it is proved beyond any question that bismuth does not adhere to the surfaces of either gastric or duodenal ulcers, should it adhere to ulcers in the large bowel?'

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THE INTESTINAL PROTOZOA OF NON-DYSENTERIC CASES

BY

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I. INTRODUCTION

While working at the Liverpool School of Tropical Medicine on the protozoa of patients suffering from dysentery, we have had the opportunity of examining also a number of patients for intestinal protozoa who entered hospital for diseases other than dysentery. The present paper gives the results of the examination of 250 such non-dysenteric patients, and the work is being continued. The diseases for which the patients entered hospital were very varied, including wounds, malaria, gastric troubles of various kinds, throat and ear complaints, appendicitis, hernia, haemorrhoids, and many others. We have not included in our results any examinations of

patients who entered hospital for dysentery. The stools were examined for intestinal protozoa in the usual way,* and the following results were obtained :—

II. GENERAL RESULTS

Total number of patients examined ... 250

Number of patients having protozoal infections 75 = 30 %

The following table shows the composition of the 75 positive cases :—

TABLE I.

	No. of cases	Percentage of all cases	Pure infection	Mixed infection
<i>Entamoeba histolytica</i>	20	8.0	5	15
<i>Entamoeba coli</i>	48	19.2	34	14
<i>Giardia</i> (= <i>Lamblia</i>) <i>intestinalis</i>	20	8.0	14	6
<i>Cbilomastix</i> (= <i>Tetramitus</i>) <i>mesnili</i>	5	2.0	1	4
<i>Trichomonas hominis</i>	4	1.7	3	1

THE DOUBLE INFECTIONS WERE :—

E. histolytica and *E. coli* in 8 cases

E. histolytica and *Lamblia* in 3 cases

E. histolytica and *Trichomonas* in 1 case

E. coli and *Tetramitus* in 2 cases

E. coli and *Lamblia* in 1 case

THE TRIPLE INFECTIONS WERE :—

E. histolytica, *E. coli* and *Tetramitus* in 1 case

E. histolytica, *E. coli* and *Lamblia* in 1 case

THERE WAS ONE QUADRUPLE INFECTION, VIZ :—

E. histolytica, *E. coli*, *Lamblia* and *Tetramitus*

NUMBER OF EXAMINATIONS

There is at present very little published evidence indicating how many examinations of an infected patient's stool are necessary before his infection can be discovered with certainty. We give in another paper in this number (p. 411) a statistical examination of evidence we have obtained upon this point in the case of patients suffering from dysentery. The significance of those figures is there fully discussed. Here it is sufficient to say that the results show the desirability of making at least three examinations of each patient

* Vide p. 412 of this Volume.

before giving any opinion regarding the infection of those found negative in these examinations. The difficulties of collection in the case of these non-dysenteric patients, where examination of the stools of the patients was no part of the regular work of the hospital, prevented us from obtaining three examinations in every case. The following are the numbers of examinations actually obtained :—

250 patients were examined at least once.
 155 patients were examined at least twice.
 74 patients were examined at least three times
 41 patients were examined at least four times.
 37 patients were examined at least five times.
 28 patients were examined at least six times.

As only 74 patients were examined three times, it is certain that the number of infections given in our table is too low. We ought to say that *at least* the given number of infections occurred. We can, perhaps, give an approximate idea by how many our positive cases would have been increased if we had examined each one twice, and also if we had examined each one three times.

We know from data given in Table II that of the 155 patients examined twice, nine new infections were found on the second examination. At this rate the 95 patients who were only examined once should have produced five or six new infections in the second examination, making a total of 80 positive cases and raising the percentage of positives to 32 per cent. instead of 30 per cent. as at present. Besides raising the total number of positives, this second examination would have added on the same assumption new protozoa in cases which were already positive, and thus increased the number of double and triple infections. No new infection was found on the third examination, though new protozoa were found in patients who already had an infection. We found, indeed, that both second, third, fourth, and in rare cases subsequent examinations, added new protozoa, usually *Entamoeba coli*, which is apparently the most sporadic in its occurrence. It follows that though the third and subsequent examinations rarely add an entirely new infection, yet the percentage of a particular protozoon, usually *E. coli*, and along with it the number of double or triple infections may be somewhat increased, and, of course, the number of pure infections correspondingly lowered.

The actual figures, showing how (a) the entirely new infections, (b) the findings of *E. coli*, (c) of *E. histolytica*, and (d) of *Lamblia* increased with repeated examinations are given in Table II.

TABLE II.

No. of patients examined	No. of examination	(a)		(b)		(c)		(d)	
		Infections of any sort found	Additional new infections	Infections of <i>E. coli</i> found	Additional infections of <i>E. coli</i>	Infections of <i>E. histolytica</i> found	Additional infections of <i>E. histolytica</i>	Infections of <i>Lamblia</i> found	Additional infections of <i>Lamblia</i>
250	1st	65	—	32	—	17	—	14	—
155	2nd	74	9	43	11	19	2	20	6
74	3rd	74	—	44	1	20	1	20	—
41	4th	75	1	46	2	20	—	20	—
37	5th	75	—	47	1	20	—	20	—
28	6th	75	—	47	—	20	—	20	—
27	7th	75	—	48	1	20	—	20	—

Of the eleven new infections of *E. coli* found on the second examination, only six were found in men who had previously been entirely negative. The remaining five went to swell the number of mixed infections, some other protozoon already being present.

DISCUSSION OF GENERAL RESULTS

Before proceeding to make certain comparisons which suggest themselves, it will be well to emphasise the broad general result that in a considerable percentage of non-dysenteric patients infections of intestinal protozoa are present. This result might perhaps have been expected in the case of *E. coli*, which is well known to be harmless and widespread. That so many infections should occur in non-dysenterics of *E. histolytica*, *Lamblia*, *Trichomonas* and *Tetramitus*, one of which certainly, and all of which possibly cause forms of dysentery, is a fact of some significance.

On account of their pathogenic importance, the infections of *E. histolytica* are of greatest interest. We wish we could have examined many more patients so as to have more reliable figures on this point. It is, however, highly significant that so many as

8 per cent. of a mixed population of non-dysenteric cases should be carriers of amoebic dysentery. The figures become even more significant when we consider that more than half of the 250 patients examined have never been out of strictly temperate regions, and therefore presumably have not been exposed to infection by *E. histolytica*, or only in the very slightest degree. In Table VII, p. 370, it is shown that of the 250 patients examined, only 123 had been in tropical or sub-tropical regions. If we consider only these 123 who have been in districts where *E. histolytica* is prevalent, the percentage of carriers of amoebic dysentery among them becomes 15·4. That this high percentage of positive cases should exist among those who have been exposed to infection and who are not suffering from dysentery is, we consider, perhaps the most striking result of our investigations. It is confirmed by the results of Dobell (1916), who examined 200 patients who had come from Gallipoli and Egypt, and had therefore been in an area where *E. histolytica* is endemic. Of these, 110 were non-dysenteric cases, and therefore strictly comparable with our 123 patients whose infections have just been given.

	Dobell	Smith and Matthews
Number of non-dysenteric patients from an area where <i>E. hist.</i> is endemic	110	123
Percentage of <i>E. histolytica</i> carriers	11·8 %	15·4 %
Number of these patients suffering from intestinal complaints	40	21
Percentage of <i>E. histolytica</i> carriers in these ...	5 %	4·8 %
Number suffering from non-intestinal complaints...	70	102
Percentage of <i>E. histolytica</i> carriers in these ...	15·7 %	17·6 %

Of our twenty *E. histolytica* cases, three had a history of dysentery, and are therefore what Dobell has called convalescent

carriers. Seventeen had no history of dysentery, and are what he has termed contact carriers. We believe that no such large number of 'contact carriers' has previously been recorded.

No separate records for non-dysenteric cases are given by Jepps (1916), but she finds 7.75 per cent. of 426 patients are carriers of *E. histolytica*. The greater proportion of these were found among dysenteric cases, and therefore the percentage of carriers of *E. histolytica* among the non-dysenteric cases must have been smaller than 7.75 per cent. All these men had been in an area where *E. histolytica* is endemic, and all were intestinal cases, so that among a certain number of non-dysenteric patients with intestinal complaints from an area where *E. histolytica* is endemic she found a percentage of *E. histolytica* carriers smaller than 7.75 per cent. It was, in fact, probably quite near our own 4.8 per cent. and Dobell's 5 per cent. in a strictly similar category.

Barratt (1916) records among forty-one non-dysenteric patients who have been exposed to infection by *E. histolytica* no cases of carriers of *E. histolytica*. This result we cannot understand, unless it was due to an insufficient number of examinations.

We will next compare our figures with those obtained by the examination of patients suffering from dysentery, and will refer to two sets of results: (1) those given by Wenyon (1916) from an examination of 556 cases of dysentery, (2) Liverpool results given in another paper in this journal (p. 411) from an examination of 910 cases of dysentery.

TABLE III.

	Wenyon's dysenteric cases	Liverpool dysenteric cases	Our own non-dysenteric cases
			From Table I, col. 2
<i>Ent. histolytica</i>	10.8 %	10.3 %	8.0 %
<i>E. coli</i>	39.0 %	25.4 %	19.2 %
<i>Lambli</i> a	16.0 %	18.6 %	8.0 %
<i>Tetramitus</i>	0.5 %	2.7 %	2.0 %
<i>Trichomonas</i>	1.6 %	1.2 %	1.7 %

The figures for non-dysenteric patients show a smaller number of infections for each of the common intestinal protozoa. This was perhaps to be expected and it would seem more worthy of note that the number of parasitic infections should so nearly approach that found in dysenteric patients. Especially is this the case with *E. histolytica*, where the percentage of infections is quite of the same order of magnitude as that found in dysenteric patients.

A very brief reference to some results which may afford comparison with our own is to be found in a paper by Hall (1916). The paper is mainly concerned with the bacteriology of paratyphoid, but the following incidental reference is made to intestinal protozoa. 'Further we had found that 10 per cent. of the paratyphoid cases showed a heavy infection with *E. histolytica*, 22 per cent. with *E. coli*, and in the last series 9 per cent. with *Lambia*.' These figures for a special class of patient are similar to those found by us for patients of all classes.

The number of double infections is rather small to draw safely any deductions from their composition. It may, however, be noted that the commonest double infection is *E. histolytica* and *E. coli*. This is also the case in the very much larger series of results obtained from dysenteric patients both by Wenyon and in this laboratory.

III. SPECIAL ASPECTS OF THE RESULTS

A. THE RELATION OF THE INFECTIONS TO THE DISEASES SUFFERED BY THE PATIENTS

We thought it of some value, considering that the intestinal protozoa would naturally be expected to be connected with diseases of the intestine, to divide the patients examined into two classes, according as they entered hospital suffering from intestinal or from non-intestinal diseases. The results are given in Table IV.

It is, of course, undesirable to lay too much stress upon figures obtained from so small a number as forty-eight, the number of intestinal cases examined, but it is at least of some significance that the total percentage of infections is not greater in the case of the patients suffering from intestinal complaints, but rather less. As far as any stress can be laid upon such small numbers, this is true of

TABLE IV.

Patients suffering from intestinal complaints				Patients suffering from non-intestinal complaints			
Number of cases examined 48				Number of cases examined 202			
Protozoal infections found in 12 = 25.0 %				Protozoal infections found in 63 = 31.2 %			
No of cases				No. of cases			
<i>E. histolytica</i>	1	=	2.1 %	<i>E. histolytica</i>	19	=	9.4 %
<i>E. coli</i>	2	=	4.2 %	<i>E. coli</i>	46	=	22.8 %
<i>Lambli</i> a	6	=	12.5 %	<i>Lambli</i> a	14	=	6.9 %
<i>Tetramitus</i>	1	=	2.1 %	<i>Tetramitus</i>	4	=	2.0 %
<i>Trichomonas</i>	2	=	4.2 %	<i>Trichomonas</i>	2	=	1.0 %

the amoebic infections also. It is, indeed, very striking that of the twenty infections of *E. histolytica* only one should be found in a patient suffering from an intestinal complaint, and of the forty-eight infections of *E. coli* only two should be in the same category.

B. DISTRIBUTION OF THE INFECTIONS BETWEEN PATIENTS WITH PREVIOUS HISTORY OF DYSENTERY OR DIARRHOEA AND PATIENTS WITH NO SUCH HISTORY

Although none of the patients were in hospital for dysentery, yet some had a previous history of dysentery, and it was thought to be desirable to separate these out (Table V).

It may be pointed out again that the number of patients with a history of dysentery is small, and it would be unsafe to draw any detailed deductions from the results. It seems to us, however, significant and surprising that the percentage of *E. histolytica* cases among those who have had dysentery is so little greater than among those who have not had dysentery. It appears that, at any rate, a significant percentage of men become carriers of *E. histolytica* without having had any dysentery at all. If our dysenteric cases were more numerous, and the same percentages still held good, we might be able to say that the number of such carriers was little less than the number of those in whom *E. histolytica* caused dysentery. This

TABLE V.

History of Dysentery				No history of dysentery			
Number of cases examined 30				Number of cases examined 220			
Protozoal infections found in 12 = 40%				Protozoal infections found in 63 = 28.6%			
			No. of cases				No. of cases
<i>E. histolytica</i>	3 = 10.0%	<i>E. histolytica</i>	17 = 7.7%
<i>E. coli</i>	9 = 30.0%	<i>E. coli</i>	39 = 17.7%
<i>Lamblia</i>	2 = 6.7%	<i>Lamblia</i>	18 = 8.2%
<i>Tetramitus</i>	1	<i>Tetramitus</i>	4
<i>Trichomonas</i>	1	<i>Trichomonas</i>	3

deduction must be withheld for the present, however, owing to the small number of cases examined having a history of dysentery. Considering the view held by some that *Lamblia* is a cause of dysentery, we think it significant that all the *Lamblia* cases but two are found among those with no previous history of dysentery.

We also ascertained which patients had a previous history of diarrhoea, and in Table VI we compare those whose record is entirely free from either dysentery or diarrhoea with those who have had one of these ailments. We include in the first column those who have had diarrhoea in a mild form, not persistent or acute diarrhoea only. The information on this point was obtained by questioning the patients, and diarrhoea was recorded in any case in which the patient could remember having had it.

In each category we have examined over 100 patients, and therefore the figures for the main infections are probably reliable. Though both *E. histolytica* and *E. coli* are found to be somewhat more prevalent among patients with a previous history of dysentery or diarrhoea, yet the main fact to be deduced from this table is the presence of a very considerable number of infections among patients who have no history of either dysentery or diarrhoea. It is noteworthy that *Lamblia* infections are equally prevalent among patients

TABLE VI.

History of dysentery or diarrhoea				No history of dysentery or diarrhoea			
Number of cases examined 110				Number of cases examined 140			
Protozoal infections found in 35 = 31.8 %				Protozoal infections found in 40 = 28.6 %			
			No. of cases				No. of cases
<i>E. histolytica</i>	11	= 10 %		<i>E. histolytica</i>	9	= 6.4 %	
<i>E. coli</i>	24	= 21.8 %		<i>E. coli</i>	24	= 17.1 %	
<i>Lamblia</i>	9	= 8.2 %		<i>Lamblia</i>	11	= 7.9 %	
<i>Tetramitus</i>	2			<i>Tetramitus</i>	3		
<i>Trichomonas</i>	2			<i>Trichomonas</i>	2		

who have no record whatever of diarrhoea, even in its mildest form. This fact has an important bearing upon the question of whether *Lamblia* causes dysentery or diarrhoea.

C. CLASSIFICATION OF PATIENTS ACCORDING TO THE REGIONS IN WHICH THEY HAVE TRAVELLED

We obtained from the patients particulars of their residence abroad and of their travels, and in the following table are given particulars of the patients from this point of view:—

TABLE VII.

Resided or travelled in tropical or sub-tropical regions		In France and England only		In Canada and England only		In England only	
Number of cases examined 123		91		12		24	
Protozoal infections found in 40 = 32.5 %		28 = 30.8 %		2		5 = 20.8 %	
		No. of cases		No. of cases		No. of cases	
<i>E. histolytica</i>	19 = 15.4 %	1	= 1.2 %	—		—	
<i>E. coli</i> ...	23 = 18.7 %	21	= 23.1 %	2		2	
<i>Lamblia</i> ...	10 = 7.1 %	7	= 8.4 %	—		3	
<i>Tetramitus</i> ...	5	—		—		—	
<i>Trichomonas</i>	4	—		—		—	

The first column, i.e., those who have resided or travelled in tropical or sub-tropical regions, includes those who have resided for longer or shorter periods in tropical Africa, India, tropical America, Mesopotamia and in the Mediterranean region, which comprised in our records, Egypt, Gallipoli and the Salonika district. In the case of sailors there was no prolonged residence in any of these districts. In the case of soldiers the residence was usually for periods of a month to two years.

Those in the second column were soldiers, and were thus exposed to conditions quite unlike those of ordinary residence in France. They were in some cases exposed to contact with native troops from tropical and sub-tropical regions, and also with British troops who had previously been in Egypt and Gallipoli.

If we consider the results set forth in columns 1, 2 and 4 of Table VII, ignoring column 3 which contains too few cases, we see that the percentage of infections of any kind falls off a little when the patients are drawn from a temperate rather than a tropical or sub-tropical region, and another fall occurs when the region is England only.

The striking fact, however, which is brought out by this table is that the *kind* of infection differs most significantly as we pass from a tropical or sub-tropical region to a strictly temperate one. With one exception, no infection of *E. histolytica*, *Tetramitus* or *Trichomonas* has been obtained in the temperate regions of Northern France, England and Canada. A much more varied intestinal fauna is at once seen in patients who have been in tropical or sub-tropical countries, and it is from these regions almost alone that the pathogenic *E. histolytica* is obtained. The case of the patient who forms the one exception to this generalisation is discussed more fully later in this paper.* *Entamoeba coli* seems to be prevalent in all regions, both tropical, sub-tropical, and temperate, and the same may be said of *Lambliia intestinalis*.

That infections should be found at all in patients who have never been out of England, or of England and Canada, is perhaps a fact sufficiently striking to merit further notice. We have given in a

* We have lately diagnosed with certainty another *E. histolytica* case from France about which up to the time of writing this paper we had been doubtful. A note on this case is appended at the end of Section D, page 383.

previous paper, Smith and Matthews (1916), a brief account of the three infections of *Lambli*a which appear to have been obtained in England, and to these cases we make further reference in a later part of this paper. We also give later a special account of the infections of *E. coli* which have apparently been contracted in this country or in Canada.

D. DETAILED HISTORIES OF PATIENTS HAVING INFECTIONS OF *E. HISTOLYTICA*. DISCUSSION OF THESE SPECIAL CASES

Before discussing the special *E. coli* and *Lambli*a infections just referred to, we consider the cases having an infection of *E. histolytica* to be so important, and the record of examinations made so interesting, that we here give a brief account of each patient's history, and in some cases an accompanying table to show the results of our observations.

CASE 2. The patient had spent a considerable part of his life at sea, having been in the Mediterranean area for six years, and in the region of the East Indies for two years. In 1905 he returned from the East Indies to England, where he resided till 1914, when he again went to sea, but remained entirely in northern latitudes. On 26 May, 1916, he was sent into hospital suffering from gun deafness. The patient stated he had never at any time suffered from diarrhoeic conditions but was rather inclined to be constipated.

Cysts of *E. histolytica* were not found in the stools till the third examination, and although the case was followed regularly for some time, only on two subsequent examinations were they again found. Cysts of *E. coli* were found twice.

When we consider the patient's history, we think it probable that he may have contracted the infection while stationed in tropical parts of the world, and that he may have been a carrier of *E. histolytica* for several years.

The record of examinations made is as follows:—

		June							July					
Date,	1916	20	21	24	26	27	28	30	1	3	4	5	6	7
<i>E. histolytica</i> cysts ...		—	—	+	—	—	—	+	—	—	+	—	—	—

+ = scanty infection.

CASE 10. The patient, before the outbreak of war, was a fisherman in the North Sea, going out from fishing towns on the north-east coast. During 1915 and 1916 he was engaged on a vessel running between England and Port Said, but before this he had never left English waters. On one occasion while ashore in Egypt the patient had an attack of diarrhoea with four or five stools a day, but never passed any blood or mucus. He blamed the drinking water.

In June 1916 he was admitted to hospital suffering from asthma and bronchitis, and by this time the diarrhoeic conditions had disappeared.

On microscopic examination of the stools being made, they were found to contain fairly large numbers of the cysts of *E. histolytica*. On one occasion the infection was very heavy, and only on three out of the fourteen examinations made were no cysts found. Cysts of *E. coli* occurred three times, and *E. coli* amoebae were observed once. Compared with Case 2, the date when this patient contracted the infection is probably much more recent, and almost certainly when he was in Egypt. The results of our examinations are shown in the following table:—

	June									July					
Date, 1916.	22	23	24	26	27	28	29	30	1	3	4	5	6	7	
<i>E. histolytica</i> cysts	++	+++	++	+	—	—	+	++	+	—	+	++	+	+	

+ = scanty. ++ = medium. +++ = heavy infection.

Scanty infection = about 10 cysts per cover-slip preparation.

Medium infection = about 30 cysts per cover-slip preparation.

Heavy infection = above 100 cysts per cover-slip preparation.

CASE 21. The patient had spent twelve months abroad in tropical regions, five months in Mesopotamia and seven months in Egypt. He was brought back to England in June, 1916, suffering from wounds and gas poisoning. We learnt, however, that he had had dysentery six months previously, had been treated, and according to the patient's own words, had been cured. During the time he stayed in hospital he had considerable gastric trouble, with frequent looseness of the bowels.

When his stools were examined, cysts of *E. histolytica* and cysts of *Lamblia intestinalis* were found. We do not know whether the dysentery from which the patient suffered in Egypt was bacillary or amoebic, but our findings suggest the latter, in which case he must have relapsed after treatment had been stopped. Unfortunately, we were unable to examine the stools at regular intervals. The record of this untreated case is interesting, however, in that it shows fairly long periods when no cysts were found in the stools, and had subsequent examinations not been made, it might have been supposed that the cysts had disappeared. The record is as follows:—

	June					July							
Date. 1916	30	4	7	10	12	14	18	20	22	25	27	28	
<i>E. histolytica</i> cysts ...	+	—	+	—	++	—	—	—	—	—	—	—	

	August									September				
Date. 1916 ...	1	2	3	4	9	11	12	14	15	9	14	19*	21	26
<i>E. histolytica</i> cysts	—	+	—	—	—	+	+	—	—	—	—	+	+	—

* Amoebae of *E. histolytica* found.

CASE 22. This patient had lived for fifteen months in India, when he was sent to Mesopotamia where he remained for four months. He was invalided to England suffering from pleurisy. On the way home he spent three weeks in Egypt. While in India he had one slight attack of diarrhoea, but did not suffer from this complaint in Mesopotamia or in Egypt or after his return to England.

The first examination of his stools revealed the presence of cysts of *E. histolytica*. In twenty-six subsequent examinations no cysts were found. This is a remarkably interesting case, since it affords a long record of a negative period in a patient who had no treatment.

The record is as follows:—

	June	July								August			
Date. 1916	30	4	5	12	14	20	22	25	27	28	1	2	4
<i>E. histolytica</i> cysts	+	—	—	—	—	—	—	—	—	—	—	—	—

	August										September			
Date. 1916 ...	7	8	10	11	12	14	15	17	19	24	30	8	15	18
<i>E. histolytica</i> cysts	—	—	—	—	—	—	—	—	—	—	—	—	—	—

CASE 31. This patient had spent the greater part of ten years at sea, and had visited tropical parts of the western hemisphere in particular. On one voyage while on the River Plate he had a short and slight attack of diarrhoea with two stools a day. The exact date of this voyage is unknown to us, but it was certainly more than two years ago. The patient had been in England since August, 1915, and in July, 1916, was admitted to hospital suffering from chronic rheumatism. While in hospital he had no diarrhoea, but generally complained of constipation.

E. histolytica and *Lambia intestinalis* were found in the stools, and in this case the record for these parasites is so interesting that we give it for both. In particular we may point out that the

occurrence of *Lamblia* in the stools alternates to some extent with the occurrence of *E. histolytica*. Only on seven occasions out of sixty-one examinations made did these protozoa occur together. Stools were examined over a long time, and in contrast to the previous case, the record, although it has distinct negative periods, shows remarkably well the persistent occurrence of cysts in an untreated patient. The record of examinations made is as follows:—

	July														
Date. 1916 ...	12	14	17	19	20	21	24	25	26	27	28*	29	31*		
<i>E. histolytica</i> cysts	+	+	+	—	—	+	+	—	—	+	+	—	—	—	
<i>Lamblia</i> cysts ...	—	+	—	—	+	+	+	—	—	—	+	+	+	—	
	August														
Date. 1916 ...	2*	3	4	5	7	9	11*	12	14	15	16	17			
<i>E. histolytica</i> cysts	—	—	—	—	—	—	—	+	+	+	+	+	+	+	
<i>Lamblia</i> cysts ...	+	+	+	—	—	—	+	+	—	—	—	—	—	—	
	August														
Date. 1916	18*	19	21	23	24	25	26	28	29	30	31				
<i>E. histolytica</i> cysts	—	—	+	+	+	+	—	+	+	+	—	—	—	—	
<i>Lamblia</i> cysts	+	—	—	—	—	—	—	—	—	—	—	—	—	+	
	September														
Date. 1916	1	2	4	5	6	7*	8	11	12	13	14	15			
<i>E. histolytica</i> cysts	+	—	+	+	+	+	+	+	+	—	+	—	—	—	
<i>Lamblia</i> cysts	+	+	—	—	—	+	—	—	—	—	—	—	—	—	
	September											October			
Date. 1916	18	19	20	21	22	25	26	27†	28	29	30	2	4		
<i>E. histolytica</i> cysts ...	+	+	+	+	+	—	+	+	+	+	+	—	+	—	
<i>Lamblia</i> cysts	—	—	—	+	—	+	+	+	—	—	+	+	—	—	

* *Lamblia* flagellates found.

† *E. histolytica* amoebae found.

CASE 84. The patient had been at sea for many years and had visited nearly all the tropical parts of the world, including Egypt, West Indies, East Indies, and West Africa. Unfortunately, we do not know the dates of any of his voyages, and, therefore, cannot form any idea regarding the time when he became infected. Since the beginning of 1915, however, he had not been out of England except for a few months spent in France. He returned from France in July, 1916, suffering from hernia and gun-shot wounds. He never suffered from diarrhoea at any time, and while in hospital complained of constipation.

Cysts of both *E. histolytica* and *E. coli* were found in the stools, the double infection occurring in nine out of twenty examinations made. This case was untreated while under our observation, and the record for *E. histolytica* is as follows:—

					August									
Date.	1916	3	5	8	11	17	19	24	26	29	31
<i>E. histolytica</i> cysts		+	+	—	—	+	—	+	++	—	+

					September									
Date.	1916	2	5	7	9	12	14	16	19	21	23
<i>E. histolytica</i> cysts		+	+	+	+	++	++	+	+	+	+

CASE 126. This patient, a native of Canada, came to England in 1915. Early in 1916 he went to Corfu where he remained three months. He was afterwards stationed at Salonika, but returned to England in August, 1916, suffering from gallstones. On his way home he spent a month in Malta. The patient complained of one attack of diarrhoea while he was in Corfu, but the derangement was apparently slight and lasted only a week.

We were able to make only five examinations of his stools and on the second of these, cysts of *E. histolytica* and *Lamblia intestinalis* were found.

CASE 133. This patient had never been out of England till May, 1915, when he went to France. In November, 1915, he proceeded to Salonika, where he contracted malaria, and was invalided home about the end of August, 1916. The patient never had diarrhoea at any time, but was always very constipated, even during the time he was in the Balkan area.

Examination of the stools showed an infection with *E. histolytica* and *E. coli*, the cysts occurring on some occasions in very considerable numbers. While the patient remained under our observation he received no treatment for dysentery. The *E. histolytica* record is shown in the following table:—

Date. 1916 <i>E. hist.</i> cysts ...	September													
	8	12	13	14	15	16	18	19	20	21	22	23	25	26
	+	+++	++	++	-	-	-	-	+	-	+	+	-	-

CASE 163. This patient went to Egypt in December, 1914. Early in the following year he contracted dysentery. The acute stage lasted a fortnight, with the passage of twenty or more stools a day. The patient remained in bed, received castor oil and gradually got better. In May, 1915, he contracted enteric and was admitted to a hospital in Alexandria. While there a liver abscess was discovered, and the patient coughed much blood, pus and mucus. Emetine injections were administered to the extent of 12 grains. Towards the end of September, 1916, he arrived in England as a convalescent, and after remaining in hospital here a very short time, he was sent to a convalescent camp.

Stools were examined only three times, and on the second examination a fair number of *E. histolytica* cysts were found, thus showing that the patient was still a carrier.

This case is not strictly a 'non-dysenteric,' but it is included in the present report, since the patient was regarded as cured when he left Egypt, and since he was not sent to a dysentery hospital in the usual way for dysenterics.

CASE 168. The patient was in South Africa from 1899-1902, and during that time had no illness. In July, 1915, he left England for the Eastern Mediterranean, and while in the Balkans he suffered from frequent attacks of diarrhoea. These lasted a few days at a time, with seven or eight motions a day, but the patient had no treatment. He contracted malaria and in September, 1916, was invalided home.

The third examination of his stools showed the presence of *E. histolytica*, and on two successive examinations cysts were again found. Seventeen subsequent examinations were made over a period of three weeks, all of which were negative. The patient had a fortnight's treatment with 'alcresta ipecac.' tablets, ten being given every day. This amounts to 19.5 grains of emetine (one tablet contains 0.15 gr.) We have not a sufficient number of examinations after treatment had ended to say that the cure is permanent.

CASE 175. The patient had never been out of England until August, 1915, when he went to Mesopotamia. He was there nine months, was wounded and invalided to India. There he remained several months until he was sent home. He never suffered from dysentery or diarrhoea at any time.

On the first examination of his stools the cysts of *E. histolytica* and *Tetramitus* flagellates were found. Subsequently the amoebae

and cysts of *E. coli* were discovered. The patient while under our observation remained untreated for dysentery, and the record for *E. histolytica* is as follows:—

Date.	Sep.		October											
	30	3	5	7	10	12	14	17	19	21	24	26	28	31
1916 <i>E. hist.</i> cysts	+	+	++	+	++	—	+	+	+	—	++	+	+	++

CASE 177. This patient had spent many years abroad. He was in Malta from 1893-95, in Egypt in 1896, and then in India till 1899. In 1899 he went to South Africa and remained there a year. From 1900-1903 he was in Ceylon, and then returned to England. He remained in this country until the outbreak of war, when he again went abroad to France and later to Salonika. During all his residence in tropical parts he never suffered from dysentery. Occasionally he had very slight attacks of diarrhoea, but they never lasted more than a day or two at a time. In Salonika he contracted malaria and was invalided home.

When the stools were examined a fair number of cysts of *E. histolytica* were immediately found. A second examination revealed the presence also of *E. coli*. The patient was given alcresta as in Case 168, i.e., he received altogether 19·5 grains of emetine. Subsequent examinations made during a fortnight after treatment had ended were all negative.

CASE 182. This patient went to France in August, 1914. He had never been abroad before. He proceeded to Salonika in November, 1915, and remained there till August, 1916, when he contracted malaria and was sent to Malta. He was in Malta eight weeks before returning to England. He never suffered from diarrhoeic conditions at any time, but was frequently constipated.

The record of the examinations made for *E. histolytica* is as follows. The disappearance of cysts after 19th September is due to treatment which is still being continued.

					September									
Date.	1916	5	6	7	19	23	25	27	30	31	
<i>E. histolytica</i> cysts		++	+	—	+	—	—	—	—	—	

CASE 198. This patient, 19 years of age, was a native of the West Indies, and had lived there for fifteen years. Then he went to Canada, and came to England in December, 1915. In July, 1916, he went to France and remained there for three months. He returned to England suffering from wounds. He had never had dysentery and had never suffered from diarrhoea.

He remained in hospital a very short time, and was examined for protozoa only twice, and on both occasions fair numbers of cysts of *E. histolytica* were found in the stools. It is probable that he may have been a carrier for several years, as it seems most likely that he would have become infected before he left the West Indies.

CASE 200. This patient had been thirteen months in France. He had never been out of England before the outbreak of war. He had been a miner in the north of England, had no illness to report, and had never suffered from diarrhoea either in France or in this country. He was invalided home at the beginning of October, 1916, suffering from wounds.

He remained in hospital a short time, and only three examinations of stools were made, but on two of these examinations *E. histolytica* cysts were found in considerable numbers.

This case is specially interesting, as it affords the only example in the present records of an infection of *E. histolytica* contracted by a patient who has not visited even the south of Europe. Considering all the circumstances, the infection was most probably contracted in France, possibly by the patient coming into contact with *E. histolytica* carriers from sub-tropical regions.

CASE 223. This patient was out of England for about eighteen months. He spent over a year in Salonika where he contracted malaria, and was invalided home in August, 1916. While in Salonika he had no dysentery, but suffered from slight diarrhoeic conditions for six days.

The patient remained under our observation for five weeks, during which time twenty-eight examinations of the stools were made. A quadruple infection was found, consisting of *E. histolytica*, *E. coli*, *Lambli*a and *Tetramitus*. In four of the first five examinations *E. histolytica* cysts were observed. Treatment with alcresta tablets extended over seventeen days, amounting to 24 grains of emetine, and all examinations after the fifth were negative for *E. histolytica*.

CASE 229. This patient left England for the first time in April, 1915. He contracted dysentery on Gallipoli and was invalided to Egypt, where he remained in hospital a fortnight, and received three injections of emetine. Subsequently he contracted malaria in Egypt and again entered hospital, remaining there for three months. In March, 1916, he proceeded to Salonika. A malarial relapse occurred, and he was invalided home in August, 1916. The patient had no dysentery or diarrhoea after the first attack on Gallipoli.

A triple infection was found consisting of *E. histolytica*, *E. coli* and *Lambli*a. The stools were examined twenty-seven times over

a period of five weeks. In the first four examinations the cysts of *E. histolytica* were found. Thereafter they disappeared, as the patient was given a course of alcresta tablets during seventeen days, amounting to 25 grains of emetine altogether.

CASE 231. This patient was away from England for less than a year. He spent nine months in Salonika and one month in Malta. In Salonika he contracted malaria, and returned to England in August, 1916. He never had dysentery or diarrhoea at any time in his life.

He was under our observation for five weeks, during which time his stools were examined on twenty-eight occasions. Cysts of *E. histolytica* and *E. coli* were found. The former occurred in large numbers on the first four examinations. Alcresta tablets were given over a period of seventeen days, amounting to 22 grains of emetine altogether. By the seventh examination *E. histolytica* cysts had disappeared, and all subsequent examinations were negative.

CASE 232. This patient left England in September, 1915. He arrived in Salonika in November and remained there till August, 1916, when he returned to England. In Salonika he contracted malaria, but never at any time suffered from dysentery or diarrhoea.

On the first examination of his stools a scanty infection of *E. histolytica* was found to be present. Examinations were continued for seven weeks, thirty-two examinations in all being made. All subsequent to the first proved negative for *E. histolytica*, and since this patient had no emetine administered, the case is particularly interesting. It resembles Case 22 in that there was apparently a disappearance of cysts from the stools in an untreated patient.

CASE 234. This patient was out of England for one year during which time he was stationed at Salonika. There he contracted malaria and returned to England in August, 1916. He had no dysentery or diarrhoea. The patient was under our observation for five weeks, and during that time twenty-five examinations of his stools were made.

An infection with *E. histolytica* and *E. coli* was found. The cysts of the former occurred on the first five examinations, and thereafter disappeared as a result of treatment with alcresta. This was given for seventeen days, a total of 24 grains of emetine being administered.

In considering the twenty cases in which infections of

E. histolytica were found, we may here point out that thirteen of them remained untreated, while to the other seven emetine was given in the form of alcresta tablets. Although this paper is not primarily concerned with the results of treatment with alcresta, a subject which is considered in another paper in this number (p. 397), it may be mentioned that at least five of the patients to whom the drug was administered have been under observation for a sufficiently long period after treatment had stopped to suggest that a cure had probably been established in each case, viz. :—Cases 177, 223, 229, 231 and 234.

Greater interest, however, is attached to the thirteen patients having *E. histolytica* who had no treatment for dysentery during the time they came under our observation. Of these thirteen patients, five, viz., Cases 126, 163, 198, 200 and 232, were examined only a few times, either because the stools were not procurable or because the particular complaint from which the patients suffered necessitated their remaining in hospital only for a very short time. Eight untreated *E. histolytica* cases remain in which the stools were examined frequently for a considerable time. These Cases are 2, 10, 21, 22, 31, 84, 133 and 175, and to each case a table is appended which shows the presence or absence of *E. histolytica* cysts in the stools on the dates on which they were examined. We endeavoured as far as possible to make daily examinations, but various difficulties rendered this impossible. Thus while the records of Cases 10, 31, and 133 are particularly good from this point of view, the regularity of examination in such a case as 21 is not so good. This is all the more unfortunate, since we wished to determine whether the cysts of *E. histolytica* disappear from the stools of untreated patients, and also to determine how many negative examinations an untreated case can give with daily examinations over a considerable period. On these points it is unsafe to draw any general conclusions, for the evidence from the small number of cases at our disposal is insufficient. All we can do is to refer to particular records. In Case 21 it will be observed that two negative periods occur, the first extending over a fortnight and the second over a month, but these periods, particularly the second, might have been considerably reduced had daily examinations been made. A fortnight's negative period again occurs in Case 31, where examina-

tions were made almost daily, and in this particular case it might be almost correctly deduced that a negative period of at least two weeks actually did occur, during which no cysts or amoebae were found in the stools. That this cannot be regarded as general, however, is obvious from a survey of the other records given, where a marked persistence of the cysts is clearly evident.

With regard to patients clearing up without treatment, we can only refer to Cases 22 and 232. In both there is a long period during which no cysts or amoebae were found, and one is tempted to conclude that a 'cure' without treatment had occurred, but this is immediately open to the objection that nothing is known of the presence or absence of cysts on the dates when no examinations were made. In spite of this, however, the two records are extremely remarkable.

Finally we may briefly refer to the fact that in these carriers of amoebic dysentery—most of them of fairly long standing—the vegetative form of the parasite was very seldom observed. This was to be expected, since none of the patients suffered from the acute stage of the disease while they were being examined.

The stools of these twenty carriers were examined in all 395 times, and *E. histolytica* was found on 127 occasions, i.e., approximately once in every three examinations (1: 3.1 exactly). From this ratio alone it may be deduced that either many shorter or several longer negative intervals occurred in the records, since on this assumption alone can the large total number of negative examinations be explained. The above figures, however, include examinations of treated patients who in most instances had negative periods of considerable extent during and after treatment. Confining our attention to the thirteen untreated patients, we find that 235 examinations were made of their stools and *E. histolytica* was found 100 times. The ratio of positive to total examinations becomes in these untreated cases 1: 2.35. This ratio indicates that on the average three examinations are necessary before the presence of *E. histolytica* in an infected patient can be demonstrated with certainty. This is an additional confirmation of the results mentioned earlier in the paper as to the desirability of at least three examinations being made of the stools of all patients suspected of harbouring *E. histolytica*.

It is clear also that the examinations should be sufficient in number and spread over such an interval as to prevent the possibility of even the extreme cases with the long negative periods escaping notice. Since we have established the occurrence of a negative period of a fortnight in an untreated *E. histolytica* case, it would seem desirable that the three examinations should be spread over a period longer than a fortnight in order to make the possibility of missing a case of *E. histolytica* still more remote.

In the 395 examinations of our twenty cases of *E. histolytica* the cysts occurred 126 times and the amoebae only three times.

CASE 116. This patient had spent twelve months in France, and in August, 1916, was invalided home suffering from shell shock. He had never been out of England before the outbreak of the war. Before he joined the Army, he had been engaged as a miner in Yorkshire. At no time in his life had he suffered from dysentery or diarrhoea.

The patient remained under our observation about three weeks during which time seven examinations of his stools were made. On four occasions the cysts of *E. coli* were found in considerable numbers. On five of the seven examinations made there were observed numbers of unusually small cysts which in many respects suggested cysts of *E. histolytica*. The number of nuclei varied from one to four, and even in the fresh condition chromidial blocks could sometimes be discerned. The cysts, however, measured only 7μ or 8μ in diameter, and this fact, together with the presence of a fairly large amount of peripheral chromatin in the nucleus and a relatively large karyosome, made a definite determination extremely difficult. Recently, stained preparations of the cysts were submitted to Prof. Warrington Yorke and Mr. Clifford Dobell, and both these authorities were of the opinion that the cysts, in spite of their small size, were those of *E. histolytica*. We are much indebted to both these gentlemen for their help in the diagnosis.

This case is the one referred to in the footnote on p. 371. It affords an additional record of an infection of *E. histolytica* apparently contracted in France, and increases our number of such cases from one to two. This case has been diagnosed too recently to enable us to include it in any of our tables as an *E. histolytica* infection.

**E. DETAILED HISTORIES OF PATIENTS HAVING INFECTIONS
OF *ENTAMOEBA COLI* APPARENTLY CONTRACTED IN ENGLAND
OR IN CANADA**

Two patients, Cases 14 and 37, who had never been out of England, came under our notice with an infection of *E. coli*.

CASE 14. The patient was in hospital suffering from a gastric ulcer. He complained of periods of diarrhoea alternating with periods of constipation.

A small infection of *E. coli* was found on the first and only examination we were able to make.

CASE 37. The patient was a sailor, but never touched at any ports other than those of the United Kingdom. For nearly two years he had been somewhere in the North Sea. He was admitted to hospital suffering from enteritis and at the same time complained of slight diarrhoeic conditions.

Amoebae of *E. coli* were found on the first examination of his stool. Nine subsequent examinations were made, and on four of these occasions the cysts were found.

Two patients who had visited Canada were found to have infections with *E. coli*.

CASE 36. This patient was in hospital suffering from a bruised foot. He had no diarrhoea.

On the two occasions when examinations of his stools were made, amoebae of *E. coli* were found. The first examination also demonstrated the presence of cysts.

CASE 194. This patient had been at sea for a year and had once crossed the Atlantic to Halifax. He had no history of diarrhoea, and was in hospital suffering from a twisted ankle.

Cysts of *E. coli* were found in all the four examinations made.

These two cases are quite comparable to the two previously described, with this difference that the patients had been to Canada.

Extended observations have been made on eight patients having an infection of *E. coli*. In seven of these cases negative periods varying from a week to over three weeks, with almost daily examinations, have been found. The record of Case 177 showed an interval of twenty-five days during which seventeen negative examinations for *E. coli* were made. In Case 229 a negative period of twenty-four days occurred, during which interval nineteen examinations were made. In both these cases the infection was small, as is often the case in *E. coli* infections, but, nevertheless, the records indicate the fallacy of concluding that a patient has lost an

E. coli infection even when many successive negative examinations are recorded. In Case 22 a few cysts of *E. coli* were found on the first examination, and although there were 26 subsequent examinations of stools over a period of about eleven weeks, cysts were not again observed. It is not unlikely, however, that, had more frequent examinations been made, cysts would again have occurred, and therefore, although it seems probable, we cannot definitely assert that the infection had entirely disappeared. We have to bear in mind also the apparent irregularity of distribution of cysts of this amoeba in faeces, and, in scanty infections in particular, this consideration may always affect any statement regarding the length of time during which no cysts are found in an *E. coli* case. In cases having a fairly heavy infection, the negative periods we have found do not generally exceed a week or ten days. In Case 223 the *E. coli* infection, which proved fairly heavy, was not discovered till the seventh examination. This was followed by a negative period of a week, but thereafter there were frequent recurrences of the cysts.

The stools of the twenty-eight patients having an infection of *E. coli* were examined 303 times altogether, and on 111 occasions the parasite was found, generally in the cyst stage, i.e., approximately once in every three examinations (1 : 2.7). This ratio indicates that at least three examinations are desirable before an opinion can be expressed whether a patient is infected with *E. coli* or not. This has been shown to be true also for *E. histolytica*, where the ratio was 1 : 2.35. In point of fact, the first three examinations provided us with all the cases of *E. histolytica* which we found, but only forty-four of the forty-eight *E. coli* cases were demonstrated by the end of the third examination. This confirms what we have found in examining dysenterics, that *E. coli* is much less regular in its occurrence than *E. histolytica*. The figures obtained from six untreated cases having both *E. coli* and *E. histolytica* infections support this view. These six cases were examined 102 times in all, and on 59 occasions *E. histolytica* was found, while *E. coli* occurred only 24 times.

In comparison with the small number of times when *E. histolytica* amoebae were found, we might here note that the 303 examinations of the forty-eight cases of *E. coli* showed that the amoeboid stage of this parasite was found in the faeces 24 times, while the cysts were present on 105 examinations.

F. DETAILED HISTORIES OF PATIENTS WHO HAVE NEVER BEEN OUT OF ENGLAND AND WHO HAVE INFECTIONS OF *GIARDIA* (= *LAMBLIA*) *INTESTINALIS*

CASE 6. The patient, a fisherman, had never been away from England or from English waters except for two days which were spent in Holland. We are thus inclined to include him in the above category. He entered hospital suffering from cardiac and gastric trouble, but he had never suffered from diarrhoea.

Two examinations of his stools were made, and on both occasions cysts of *Lambli*a were found in large numbers, on the first a few flagellates also being present. As the patient soon recovered from his ailment, and was discharged from hospital, we were unable to follow the case further.

CASE 15. The patient entered hospital suffering from gastric ulcer. He had previously suffered slightly from diarrhoea, but had no such tendency when he came under our observation. He joined the army in August, 1914, but his gastric trouble had prevented him from being sent abroad, and he had never left England up to the time of our examinations.

While under treatment we were able to make one examination of his stool, in which were found cysts of *Lambli*a in fair numbers.

CASE 238. This patient was called up to serve with the colours on June 5th, 1916. He had six weeks training and then entered hospital to be treated for diarrhoea. Before joining the Army he was a farmer in Shropshire, and had never at any time been out of England. Indeed he had never been further from his own farm than the small market town of that district, which was only four or five miles from his home. For twelve years past he had suffered from attacks of diarrhoea at irregular intervals, and during the more acute attacks had passed four, five, or even more stools daily. The diarrhoea often followed exposure to cold.

He remained under our observation a considerable time, and the first examination showed that he had a *Lambli*a infection, which was soon found to be a heavy one. The complete record of his examinations is as follows:—

		July						
Date. 1916 ...	10	12	13	14	17	18	19	
<i>Lambli</i> a cysts ...	+	+++	++	++	++	++	+++	

		July					
Date. 1916		20	21	22	24	25	26
<i>Lamblia</i> cysts		+++	+++	++	++	+++	++

		July				August				
Date. 1916		27	28	29	31	1	2	3	4	5
<i>Lamblia</i> cysts		+	—	—	—	—	—	—	—	—

		August							
Date. 1916		7	8	9	10	11	16	17	18
<i>Lamblia</i> cysts		+	+	+	+	+	+	++	+

+ = scanty infection of *Lamblia* cysts.

++ = medium infection of *Lamblia* cysts.

+++ = heavy infection of *Lamblia* cysts.

Though the infection from July 12th to 26th was a heavy one, yet a negative period of nine days followed. Afterwards cysts again appeared in the stools, and persisted until the patient passed from under our observation.

His case is of particular interest, as it raises the question of the causation of diarrhoea by *Lamblia intestinalis*. At first sight one is inclined to conclude that his diarrhoea was caused by the *Lamblia* infection and that this is a case of lambliasis contracted in England. When, however, we consider the results set forth in Table VI of this paper, and see that infections of *Lamblia* are as common in patients who have no record of diarrhoea as in those who have, it becomes necessary to hesitate before stating as a fact that the diarrhoea in this patient was caused by the *Lamblia* infection. It is clear that if a patient is subject to diarrhoea from some other cause the cysts and flagellates of *Lamblia* will be likely to occur in such diarrhoeic stools. It is perhaps useless to speculate further, as we have no prolonged observations as to the connection between the occurrence of *Lamblia* and the condition of the stools. When all the facts are considered it becomes a matter of extreme difficulty to say with certainty that *Lamblia* is the cause of diarrhoea in any

particular case. It is much more difficult than in the case of *Entamoeba histolytica*, for there the severity of the symptoms, the finding of the amoebae in the blood and slime of a dysenteric stool, the ingestion of red blood corpuscles by the amoebae, and many other facts, render it very probable that *E. histolytica* is the primary cause of the dysentery, in spite of the fact that we are now finding a considerable number of cases in which an infection of *E. histolytica* has not caused dysentery, and has in fact appeared to be quite harmless.

Continued observations have been obtained for only five out of the twenty patients infected with *Lamblia*. Of these five, Case 238 has just been described and his detailed record given. In Case 31 the details of the *Lamblia* infection are given with those of *E. histolytica* on p. 375. In Case 31 two distinct negative periods occurred extending just under a fortnight, and in Case 238 one such negative period occurred. Since in these cases examinations were made almost daily, it might have been concluded that the infection had disappeared, had not subsequent examinations shown conclusively that *Lamblia* was still present.

In Case 21, whose record for *E. histolytica* has been given in p. 374, *Lamblia* cysts were found until July 20th. Thereafter they disappeared, not being found again in the eighteen examinations which followed. This would seem to point to a permanent disappearance of the infection, but this conclusion is scarcely justifiable without more frequent examinations than we were able to make. In the other two cases, 223 and 229, the cysts occurred with almost uninterrupted regularity.

The number of examinations made of the twenty patients having *Lamblia* infections was 198. *Lamblia* was found on 113 occasions, i.e., in the ratio 1 positive : 1.75 examinations. The ratio of positives is somewhat higher than in untreated *E. histolytica* cases (1 : 2.35) or in *E. coli* cases (1 : 2.7), and this agrees with our experience from a large number of dysenteric cases, in which we find that *Lamblia* appears with greater regularity than *Entamoeba histolytica*, or especially *E. coli*. Out of the 113 times when *Lamblia* was found the cysts occurred on 108 occasions and flagellates occurred on 13 occasions.

G. ORGANISMS FOUND OTHER THAN PROTOZOA

In addition to the common intestinal protozoa which we found among the patients whose stools we examined, we think it may be of some interest to record the occurrence of cyst-like bodies which were observed in the faeces of seven of the total number of cases examined. These were the vegetable organisms described by Wenyon (1915), and to which the name 'I body' has been given. The infection was pure in four cases, but in the other three cases the organism occurred with various protozoa. The infection was occasionally fairly heavy, and in two cases was remarkably persistent. In four cases the patients had a previous history of diarrhoea, while in the remaining three cases there was none. We are not in a position to remark on the pathogenicity of these organisms, but it may not be without some significance that six out of the seven patients infected had been in the Mediterranean area. In the remaining case the patient had spent a year in France.

Finally there remains to be recorded the occurrence of the eggs of *Trichocephalus dispar* (*Trichiurus trichiura*) in four cases. The infection was always small. Three of the patients concerned had been to sub-tropical parts of the world. One of the infections occurred, however, in a sailor who had never been out of home waters.

In conclusion, we wish gratefully to acknowledge the help we have received from various sources. To Professor Stephens we are indebted for introducing us to the hospital from which we have obtained the material for our investigation and for his continued interest in the work. We wish also to thank Dr. Abram, Mr. Thelwall Thomas and Mr. Jeans for permission to work in the wards under their charge, and to the sisters and nurses of these wards we express our thanks for their kindness and attention in the supply of the specimens examined.

To Mr. Clifford Dobell we are indebted for suggesting the line of investigation we have followed in this paper, and finally we are very grateful to Dr. D. L. Mackinnon and Mr. H. F. Carter who have both given their assistance in the actual microscopic examinations.

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REMARKS ON THE SPIROCHAETES OCCURRING IN THE FAECES OF DYSENTERIC PATIENTS

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At the commencement of the present year, while performing, in the laboratory of the Liverpool School of Tropical Medicine, routine examinations of the stools of soldiers suffering from dysentery, actively moving spirochaetes were occasionally detected in the fresh preparations. Owing to the ease with which these minute organisms may be overlooked in such preparations, particularly when the examinations have to be conducted with some rapidity and without employing the highest powers of the microscope to any great extent, the percentage of infected cases appeared low. Accordingly, at the suggestion of Professor Stephens, advantage was taken of the plentiful supply of material available, to make a systematic examination of stained preparations of faeces. The results of this investigation and of a comparative study of the faeces of one hundred non-dysenteric patients form the subject of this article.

The presence of spirochaetes in the stools of both sick and healthy persons has been noted on several occasions during recent years. They have been previously observed in the faeces of patients suffering from amoebic dysentery, cholera and sprue, and Le Dantec in 1903 even cited them as the causative organisms in a case of dysentery said to have been developed in France. However, such records are for the most part isolated, and apparently no serious attempt has been made to determine the percentage of infected individuals—either in healthy or diseased people. Certainly J. G. and D. Thomson (1914) examined the dejecta of numerous persons, both normal and diseased, for spirochaetes, and concluded that a certain proportion of the cases were infected with Werner's two species—*S. eurygyrata* and *S. stenogyrata*. Fantham (1915), also, has given a good account of the morphology of spirochaetes

occurring in the faeces of soldiers suffering from dysentery or diarrhoea, but no numerical data, based on stained specimens, have so far been made.

The validity of many of the species of faecal spirochaetes encountered and described by different authors appears doubtful, and, according to Fantham, it is probable that only one species—*S. eurygyrata*, Werner—should be recognised.

For the purpose of the present investigation rapidity was an important factor, and the careful fixation and staining necessary for morphological study was not required. After the fresh preparations had been examined for pathogenic protozoa, therefore, the cover-slips were pushed off the slides and the smears thus obtained fixed by heat. They were then stained with Giemsa or gentian violet.

The examinations (under a $\frac{1}{12}$ -inch objective and No. 2 ocular) were made as thoroughly as possible in spite of the somewhat limited time available, and from twenty to thirty minutes were spent in carefully searching the negative or apparently negative cases. In all, smears from the stools of 554 patients admitted into hospital for dysentery or related diseases were examined, and of these 313, or 56·5 per cent., showed spirochaetes. Whenever possible, additional samples of the faeces of those proving negative on the first examination were procured and examined after an interval of a week or two; as many as four or five examinations of one patient were sometimes made. Unfortunately, however, a comparatively large number of such cases was not available for further observation, and as a relatively high proportion of the 'first examination negatives' which were obtained for subsequent examination proved positive, the percentage given above is certainly too low. The details of the observations made are as follows:—

	Cases	Positive	Negative
Examined once	289	180	109
Examined twice	211	115	96
Examined three times	49	16	33
Examined four times	4	2	2
Examined five times	1	—	1
Total	554	313	241

That the cases examined more than once show a rather lower percentage of positive infections (50·2) is only to be expected, since many of those proving positive as a result of the first examination were not inspected again. Thus of the 265 cases subjected to two or more examinations, only 75 had been previously shown to harbour spirochaetes; but of the remaining 190 no less than 58, or 30·5 per cent., proved positive subsequently. This relatively high proportion is no doubt chiefly due to the entire disappearance at times of the spirochaetes as such from infected stools. To a smaller extent, perhaps, it may be accounted for by the comparative frequency with which scanty infections were encountered and the corresponding increased tendency to incorrect diagnosis;* particularly would this be so if only young and very small forms were present. If, however, this proportion (30 per cent.) of those negative cases which only received one examination be regarded as what may be termed 'latent positives,' the final percentage of infected cases would be slightly raised. Of the 109 negative cases referred to in the above table, therefore, about thirty-three should ultimately prove infected, and the total number of positive cases (313) should accordingly be raised to 346 and the percentages from 56·5 to 62·4.

With a view to obtaining, if possible, some evidence regarding the pathogenicity of these organisms, or at least of determining whether disorders of the alimentary canal, induced by pathogenic amoebae, favoured their existence, an examination of the faeces of a number of patients free from such parasites was undertaken.† Stained preparations were made of the stools of 100 cases contained in the Royal Infirmary, Liverpool. These men were suffering from a variety of affections, e.g., rheumatism, hernia, paralysis, bronchitis, pleurisy, neurasthenia, cardiac affections, wounds, gun-deafness, etc., etc. The following is an analysis of the examinations made:—

* In this connection it may be remarked that, although no attempt was made to determine the relative densities of the various infections, only sixty-eight of the total positive cases found (which, including those of the non-dysenterics, numbered 354) were marked as possessing heavy or moderately heavy infections.

† The stools of these patients had been previously examined for *Entamoeba histolytica* and were selected because of their entire freedom from this parasite.

	Cases	Positive	Negative
Examined once	81	33	48
Examined twice	17	7	10
Examined three times	2	1	1
Total	100	41	59

Forty-one per cent. of the cases were thus directly shown to be infected with spirochaetes. It was not possible to obtain samples of the stools of the great majority of these patients more than once, and therefore an appreciable number of 'latent positives' was probably included among the 'negatives.' Owing to this difficulty and to the comparatively small number of cases involved in the investigation, no closer estimate of the ultimate findings can be suggested. It may be pointed out, however, that of the nineteen cases whose faeces were procured for further examination, fourteen were at first declared negative, on subsequent examination three of these showed spirochaetes.

In view of these results, it would seem that spirochaetal infections of the alimentary canal are almost, if not quite, as prevalent in non-dysenteric as in dysenteric patients. The difference indicated between the proportions of infected cases in the respective classes is not so great as to allow the inference that conditions more favourable to the existence and development of these organisms occur when certain pathological aspects of the gut are present. In fact, two of the heaviest and seemingly most persistent infections noted throughout the whole investigation were found among the non-dysenteric cases; one of these patients was under treatment for ear and throat affections and the other for rectal abscess.

Fantham and others have remarked upon the disappearance, from time to time, of spirochaetes from infected stools, but whether this disappearance is total or only apparent is unknown, owing to our present inability to distinguish the coccoid body or granule stage from surrounding faecal debris. Daily variation in the

intensity of the infection has also been noted, and in this investigation was occasionally marked enough to attract attention. An attempt was therefore made to determine whether such increases in the number of spirochaetes present were periodic and also to obtain information relating to the duration or persistence of the maximum infections and the average length of time elapsing between such infections. With these objects in view, eight positive cases, which could be obtained frequently, were selected and examined as often as possible during one month. At the commencement of this investigation six of these cases were scantily infected and two were 'latent positives,' but when previously examined all had shown spirochaetes, two in fairly large numbers. Six of the patients also were being treated for amoebic dysentery, and all were infected with one or more species of Protozoa. Unfortunately no definite conclusions could be deduced from the results, as throughout the whole period none of the infections increased to any appreciable extent, and one remained continuously latent.

Spirochaetal infections were, as previously indicated, frequently found mixed with single or multiple Protozoal infections of the alimentary tract. From an analysis of the records kept, however, there would appear to be no correlation between the presence of these organisms and the occurrence of any of the commoner Protozoa in the stools. Of the 554 patients admitted into hospital for dysentery, and examined for spirochaetes, 51 were infected with *Entamoeba histolytica*, Schaudinn, 123 with *Entamoeba coli*, (Lösch), and 116 with *Giardia (Lamblia) intestinalis*, (Lambl). These infections, in regard to their relations with spirochaetes, were distributed as follows:—

	Cases	Spirochaetes present	Spirochaetes absent
<i>E. histolytica</i>	51	23	28
<i>E. coli</i>	123	67	56
<i>G. intestinalis</i>	116	57	59

Thus 45 per cent. of the cases infected with *E. histolytica*, 54 per cent. of the *E. coli* infections and 49 per cent. of the *G. intestinalis* infections are positive to spirochaetes. Among the non-dysenteric cases there were four infections of *G. intestinalis* and seventeen of *E. coli*; one of the former and twelve of the latter were mixed with spirochaetes. Among these patients, therefore, no less than 70 per cent. of the *E. coli* infections were positive to spirochaetes, but the figures available are unfortunately too small for any deductions to be drawn.

In conclusion, a few remarks may perhaps be made regarding the length and agglomeration of spirochaetes as observed while the examinations were in progress. *S. eurygyrata* was stated by Werner to vary in length from 4.6μ to 7.3μ ; other authors writing on faecal spirochaetes have further separated these extremes, and Fantham has recently given from 3μ to 15μ as the variation encountered. Upwards of fifty individuals were selected at hazard and carefully measured; the range in size noted was from 2.8μ to 10.8μ , and the average length from 4μ to 7μ .

S. eurygyrata, like the blood spirochaetes, is said to commonly occur in masses and tangles, and probably this is so in heavy infections; comparatively few such masses were seen during this investigation, however, and then only when the spirochaetes were present in large numbers.

My thanks are due to Prof. J. W. W. Stephens for much helpful advice, and to Messrs. A. Malins Smith and J. R. Matthews for allowing me to select suitable material from that which they had collected for their work on the Protozoa of Non-Dysenteric Patients.

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A PRELIMINARY STATEMENT ON THE TREATMENT OF *ENTAMOEBIA* *HISTOLYTICA* INFECTIONS BY 'ALCRESTA IPECAC.'

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I. ALCRESTA IPECAC.

At the Tropical School Auxiliary Military Hospital in the end of June, 1916, the treatment of *Entamoeba histolytica* infections by emetine hydrochloride was given up almost entirely in favour of treatment with 'alcresta ipecac.'

Alcresta ipecac. is the trade name for an adsorption compound of ipecac. alkaloids with hydrated aluminium silicate (Fuller's earth). It is made up in the form of tablets, and each tablet is advertised to contain the alkaloids from 10 grs. of Rio ipecac. U.S.P. Allan (1916) calculates in his 'Clinical Notes on the Use of Alcresta Ipecac. in Amoebic Dysentery' that, 'since U.S.P. ipecac. contains as a minimum 2 per cent. total alkaloids, Rio ipecac. assaying 1.5 per cent. emetine and 0.5 per cent. cephaeline, each tablet will therefore contain as a minimum 0.15 grs. of emetine.'

It is stated of alcresta ipecac. that it 'passes through the stomach unchanged and liberates the alkaloids in the alkaline intestinal

secretions,' and it is claimed that patients taking emetine in this form suffer little, if at all, from the nausea and sickness that are so unpleasant a feature of some other forms of emetine treatment.

In America, alcresta seems to have been used mainly in cases of pyorrhoea, but Allan in the paper quoted above describes the use of alcresta in ten cases of amoebic dysentery. His experience bears out the statement as to the ease with which the drug can be taken in this form; he is of opinion, however, that emetine given hypodermically is 'twice as efficacious' as emetine given by the mouth in the form of alcresta tablets. It may be noted that the cases he was dealing with were cases of acute dysentery. Except for the cases quoted in Allan's paper, we do not know of any other published account of the treatment of amoebic dysentery with alcresta ipecac.

The experience at the Tropical School Auxiliary Military Hospital this year fully confirms the claim that emetine given in this form causes little or no inconvenience to the patient. Hypodermic treatment with emetine hydrochloride was not much used owing to the practical inconvenience in dealing with large numbers of cases. Previous to the use of alcresta at this hospital, the men were being given emetine hydrochloride tablets orally, but even where these were coated with sandarac varnish there were many complaints of nausea and vomiting. Since the use of alcresta began there has been practically no trouble of the kind.

We are not at present able to state with certainty the amount of alcresta that constitutes an effectual 'treatment,' and the length of time over which a 'treatment' should extend. In view of the great demand on the accommodation in the military hospitals, it would clearly be of much practical value to discover what is the minimum amount of alcresta that can be trusted to clear the average case from *E. histolytica* cysts, and what is the shortest time into which such a treatment can be compressed. At present we are unable to make any final statement on these points. In the beginning, the amounts given were very various in the different cases, and the whole plan was tentative; later, we adopted a standard 'course' of fourteen days' treatment with ten tablets daily, five at night and five in the morning, i.e., $1\frac{1}{2}$ grs. emetine per day for a fortnight.* Where a patient 'relapses' after such a course, he is at once put

* More recently still, a number of cases have been put on a 5 days' course of 1.5 grs. per diem.

on a second, and if need be on a third. This is our present practice. In most cases one 'course' is found sufficient; and on the whole, as will be judged from the detailed account following, the use of alcresta is justified so far by the results. The majority of the patients have responded well to the treatment, and after being subjected to a pretty severe microscopical test, have been discharged from hospital as cured.

II. MATERIAL AND METHODS

Without an exception, the cases treated were in the chronic condition of amoebic dysentery, i.e. were 'carriers.' The men were soldiers who had become infected with *E. histolytica*, mainly in Gallipoli, Salonika, Egypt, and Mesopotamia. Examination of thin films of the patients' faeces under the microscope revealed the presence of the cysts of the entamoeba. When a man was discovered to be a cyst-carrier, he was forthwith put on a 'course' of alcresta, and throughout this treatment a specimen of his faeces was examined daily in the laboratory, so that we might judge whether the drug was taking effect. These daily examinations of a large number of cases involved a great deal of additional work, but it was felt that only by this careful control could we estimate the real efficacy of alcresta in getting rid of the infection. Later on, when the value of the drug has been more widely recognised and tested, it will probably be unnecessary, as indeed it would be impossible in general practice, to make daily examinations over long periods; but during this period of trial we lay great stress on close and sustained microscopical control. We hope to be able later on to formulate some less laborious rule for general procedure.

As will be judged from what follows, it is very important when testing any emetine compound that the daily observations should not cease with the treatment, but should extend over *as long a period after treatment as is practicable*.*

* It must be pointed out that, though daily examination was aimed at, this was not always found possible in practice. For various reasons, examinations were often made only every second or third day over certain periods; the intervals were sometimes longer still. Hence, it must be understood that when we speak in this paper of 'a week's negative interval,' we do not necessarily mean a period in which six consecutive daily examinations were recorded. 'A week's negative interval' may be based on six consecutive examinations in one man, and on only three or four in another. We have not been able to do more than make an approximation to the ideal daily examination test.

III. ANALYSIS OF THE CASES

From June 22nd until November 30th, 1916, eighty-one carriers of the cysts of *Entamoeba histolytica* treated with alcresta ipecac. were examined in the way described. Three of these cases were treated in the Royal Infirmary; the rest were in the Tropical School Auxiliary Military Hospital.

For convenience, we may group these seventy-six men in the following scheme:—

- i. Cases still under treatment.
- ii. Cases that left hospital as soon as their treatment had stopped.
- iii. Cases observed after treatment.
 - A. Cases that did not relapse after a first course of alcresta.
 - B. Cases that relapsed after a first course of alcresta.
 - (a) Cases that remained negative after a second or third course.
 - (b) Cases that remained 'refractory' to continued treatment with alcresta.

These sub-divisions may now be considered separately.

i. *Twelve men whose treatment began in the middle or end of November, 1916, had not at the time of writing this been observed for a sufficiently long time to justify an opinion as to their 'cure.'* At present they have all become negative for *E. histolytica* cysts. We hope to include them in a future report.

ii. *Thirteen men who left hospital immediately treatment had stopped* (Cases I, II, IX, X, XII, XIII, XIV, XVI, XVII, XIX, XXXVI), or so soon after treatment (Cases XVIII and LX) that it was not possible to keep them under observation for a satisfactory post-treatment period. These cases all ceased to pass cysts a few days after treatment began. Cases I and XVIII, indeed, after becoming negative, passed a few cysts during treatment. But all, with the exception of XII, XIV, and XXXVI, which were discharged after shorter periods, remained negative under observation for periods of three weeks and over.

Experience shows, however, that with alcresta, as with other preparations of emetine, a negative period *during* treatment is no

real guarantee of cure; the patient may begin to pass cysts again when the inhibiting effect of the drug on the development of the amoeba has ceased. We find the majority of cases that 'relapse' in this way, do so in the first week after treatment stops; but a relapse at the end of fourteen days is not uncommon, and we have known a case to 'relapse' after twenty-four days. It is therefore to be recommended that patients should be observed for *at least* a fortnight, and if possible for three weeks, after treatment and before they are discharged 'as cured.*' This question of 'relapsing' cases is more fully discussed in a later section of this paper.

The following is a copy of the Army Council Instruction No. 1,354 of 1916 with regard to the Treatment and Disposal of Convalescents from Dysentery:—*'A patient suffering from dysentery who is transferred to the United Kingdom from an Expeditionary Force will remain in the Central Hospital to which he has been admitted at least 14 days, during which period two bacteriological and protozoological examinations of his dejecta will be carried out, with an interval of at least seven days between them.'*

It may be pointed out here that, according to this instruction, no less than seventy-three out of seventy-six cases might have been discharged from the Tropical School Hospital and the Royal Infirmary to a dysentery camp at one time or another while they were under treatment, and this whether they had really ceased to be carriers of pathogenic cysts or not. For seventy-three of seventy-six, and among them some of the most 'refractory' of the cases, have shown, under the temporary influence of alcresta, two consecutive negative examinations at a week's interval.

iii. *Fifty-six men whom it was found possible to keep under close observation for a satisfactory period after treatment.*

A. A majority, thirty-eight, showed excellent response to the treatment, and remained negative after treatment for periods

* We must explain that throughout this paper we use 'cure' with a certain reservation. In practice it is not found possible to keep the men in hospital during war-time for very long post-treatment observation. It is only in certain cases where, for reasons unconnected with dysentery, the men were detained in hospital over long periods that we were able to show that cysts were not passed during observation extending after treatment for as much as seven weeks. We should be inclined to account cases 'cured' that passed no cysts for three weeks after treatment; but so little is yet known of the life-conditions of *E. histolytica*, and of the mode of recurrence of amoebic dysentery, that it is unwise to talk too confidently about 'cures.'

varying from one week four days, to seven weeks (in the case of three men detained in hospital for reasons unconnected with dysentery).

The detailed observations on these thirty-eight cases are set out in the following table:—

TABLE I.

Cases that did not pass cysts after first treatment with Alcresta

Case No.	Treatment with Alcresta			Period of Consecutive Negative Examinations	Total No. of Consecutive Negative Examinations	No. of Consecutive Negative Examinations after Treatment
	Begun	Ended	Total Emetine			
V	June 22	July 25	15.5 grs.	July 7 to Aug. 8 ...	20	8
VII	July 21	Aug. 20	22.5 grs.	July 22 to Sept. 20 ...	39	18
XV	Aug. 22	Sept. 28	27 grs.	Sept. 2 to Nov. 23 ...	63	41
XXI	July 18	Aug. 18	31.8 grs.	Aug. 14 to Oct. 6 ...	34	30
XXII	Aug. 24	Sept. 4	9.9 grs.	Aug. 23 to Sept. 25 ...	26	17
XXIII	Aug. 23	Sept. 4	9.35 grs.	Aug. 24 to Oct. 10 ...	30	21
XXIV	Aug. 23	Sept. 4	9.9 grs.	Aug. 24 to Sept. 25 ...	26	16
XXVI	Aug. 2	Aug. 18	11.25 grs.	Aug. 7 to Sept. 20 ...	24	17
XXVII	July 28	Aug. 18	11.25 grs.	Aug. 22 to Sept. 18 ...	18	18
XXVIII	Aug. 2	Aug. 20	13.5 grs.	Aug. 5 to Sept. 20 ...	32	19
XXIX	Aug. 20	Aug. 27	5.5 grs.	Aug. 12 to Sept. 21 ...	34	22
XXX	Aug. 3	Aug. 20	19.5 grs.	Aug. 9 to Sept. 30 ...	20	11
XXXII	Aug. 3	Aug. 20	25.5 grs.	Aug. 5 to Sept. 21 ...	38	24
XXXIV	Aug. 3	Aug. 20	25.5 grs.	Aug. 5 to Sept. 21 ...	26	13
XXXVII	Sept. 5	Sept. 25	30 grs.	Sept. 7 to Oct. 17 ...	26	13
XXXVIII	Sept. 11	Sept. 28	24 grs.	Sept. 11 to Oct. 12 ...	22	9
XXXIX	Sept. 14	Sept. 30	22.5 grs.	Sept. 15 to Oct. 12 ...	22	9
XL	Sept. 11	Sept. 28	25.5 grs.	Sept. 12 to Oct. 10 ...	23	10
XLI	Sept. 11	Sept. 28	24 grs.	Sept. 15 to Oct. 11 ...	20	8
XLII	Sept. 19	Oct. 3	19.5 grs.	Sept. 19 to Nov. 9 ...	32	20

TABLE I.—Continued.

Case No.	Treatment with Alcresta			Period of Consecutive Negative Examinations	Total No. of Consecutive Negative Examinations	No. of Consecutive Negative Examinations after Treatment
	Begun	Ended	Total Emetine			
XLIII	Sept. 12	Sept. 28	25.5 grs.	Sept. 14 to Nov. 11 ...	44	33
XLIV	Oct. 5	Oct. 19	19.5 grs.	Oct. 7 to Nov. 16 ...	27	18
XLV	Oct. 3	Oct. 29	24 grs.	Oct. 6 to Nov. 24 ...	39	19
XLVI	Oct. 6	Oct. 23	19.5 grs.	Oct. 9 to Nov. 9 ...	22	12
XLVII	Oct. 16	Nov. 8	33 grs.	Oct. 21 to Nov. 23 ...	21	8
XLVIII	Oct. 5	Oct. 19	19.5 grs.	Oct. 5 to Nov. 16 ...	30	19
XLIX	Oct. 12	Oct. 26	19.5 grs.	Oct. 14 to Nov. 7 ...	20	9
L	Oct. 4	Oct. 22	19.5 grs.	Oct. 12 to Nov. 23 ...	24	18
LI	Oct. 4	Oct. 27	19.5 grs.	Oct. 10 to Nov. 10 ...	16	6
LII	Oct. 12	Oct. 29	24 grs.	Oct. 18 to Nov. 25 ... (continuing)	26	18
LIV	Oct. 12	Oct. 29	24 grs.	Oct. 16 to Nov. 24 ...	29	17
LVI	Oct. 6	Oct. 21	20 grs.	Oct. 9 to Nov. 9 ...	21	13
LX	Oct. 20	Nov. 8	27 grs.	Oct. 25 to Nov. 23 ... (continuing)	15	7
LXI	Oct. 16	Oct. 30	19.5 grs.	Oct. 20 to Nov. 24 ... (continuing)	25	17
LXII	Oct. 13	Oct. 27	19.5 grs.	Oct. 16 to Nov. 22 ...	31	21
LXV	Oct. 19	Nov. 8	28.5 grs.	Oct. 23 to Nov. 24 ... (continuing)	25	17
LXVII	Oct. 25	Nov. 12	27 grs.	Oct. 28 to Nov. 20 ...	19	9
LXVIII	Oct. 5	Nov. 18	19.5 grs.	Nov. 4 to Nov. 24 ... (continuing)	16	5

It can be shown from Table I that thirty-eight patients treated with alcresta for the first time became 'negative' on an average three days after treatment began, and showed no cysts in their faeces during a long series of consecutive examinations extending through the period of treatment and for a reasonable length of time afterwards.

Twenty-three of the thirty-eight were negative for three weeks and more after treatment, and it was possible to follow some of them for seven weeks and more. (In one of these cases, sixty-three

consecutive negative examinations were made, forty-one of them after treatment; in another forty-four, thirty-three of them after treatment).

It was not possible to follow the other fifteen cases for such long periods, owing to the demand on hospital accommodation, but the records are very satisfactory so far as they go. Nine remained negative for a post-treatment period of over two weeks; four were discharged after a period of between one and two weeks, and two which have had more than a week's observation since treatment remain negative and are still being examined daily.

None of these thirty-eight cases, then, showed any sign of 'relapse,' and the majority of them have been discharged.

A Note on Case XXIX. CASE XXIX was negative when admitted to the Tropical School Hospital, though he came with a record of 'pathogenic amoebae' from another hospital. After two negative examinations a heavy infection of *E. histolytica* cysts was observed. Six negative examinations followed. It was not until then—after he had begun a negative period unaided—that he was put on alcresta. The course was very short (only 5.5 grs.). During it he had six more negative examinations; and 22 negative examinations followed, before he was discharged as cured. That is to say, he has never been 'positive' since his third examination in this hospital. He has had no less than 34 consecutive negative examinations, and has been discharged as cured, but it is not possible to maintain with any certainty that alcresta was responsible for the clearing up in this case.

B. This section of the fifty-six cases observed after treatment contains eighteen which 'relapsed.' On four of these alcresta seemed to produce so little effect that they passed cysts almost continuously both during treatment and after it.

The incidence of 'relapses' is best brought out by a tabular comparison of the cases, where 'Positive' means that cysts were being passed in the faeces and 'Negative' that they were not.

TABLE II.

	Negative	Positive
Of 69 cases observed at the end of a first treatment	63	6 (8.6%)
Of 50 cases, hitherto negative, observed at end of 1st week after treatment	42	8 (16%)
Of 38 cases, hitherto negative, observed at end of 2nd week after treatment	36	2 (5.2%)
Of 25 cases, hitherto negative, observed at end of 3rd week after treatment	24	1 (4%)
Of 17 cases, hitherto negative, observed at end of 4th week after treatment	16	1 (5.8%)
Of 7 cases, hitherto negative, observed at end of 5th week after treatment	7	0
Of 6 cases, hitherto negative, observed at end of 6th week after treatment	6	0
Of 3 cases, hitherto negative, observed at end of 7th week after treatment	3	0

That is to say, of fifty negative cases observed *after treatment* at intervals of a week, twelve (24 per cent.) relapsed in a period of seven weeks. Or, more comprehensively, to include those cases that were positive at the end of treatment, of fifty-six cases observed after treatment a first 'course' of alcresta failed to clear up eighteen (32 per cent.).

It can be seen from the above table that relapses after treatment occur chiefly in the first and second weeks, and that is why we lay so much stress on keeping the treated case under observation for *at least* two weeks when he is taken off alcresta. While relapses seem to be unusual after the second week, nevertheless one case relapsed on the twenty-fourth day after treatment. Therefore, the cases which have been followed for three weeks and more after treatment are the only ones of whose 'cure' we can feel considerable confidence; although it is reasonable to suppose that the majority of those showing negative periods of two weeks and more are also likely to remain negative in the future. We are the more inclined to regard the two weeks' negative interval as a fairly good criterion of 'cure,' since observations carried out by Malins Smith and Matthews (1917) in this laboratory on untreated cases harbouring *E. histolytica* show in a long series of consecutive examinations only one instance where so long a period as a fortnight elapsed with no sign of a cyst in the faeces.

As indicated above, it seems easiest to consider the eighteen relapsing cases in two groups: (a) cases still amenable to further treatment with alcresta, and (b) 'refractory' cases, on which prolonged further treatment with alcresta produced no improvement.

This distinction is one of convenience and is rather artificial, seeing that some of the relapsing cases at present in (a) may eventually turn out to be 'refractory.'

(a) Fourteen cases which relapsed at the end of or after a first 'course,' but cleared up under a second or third, or show signs of doing so (see Table III).

Of cases III, VIII, XX, and XXXV it is not possible to say confidently that they were cleared of cysts when discharged, for the reason that they had to leave hospital before they had been long observed after their second 'course' of alcresta.

CASE VIII, indeed, was transferred while still passing cysts, and we were unable to follow him further.

TABLE III.
'Relapsing' Cases.

Case No.	Treatment with Alcresta			Period of Consecutive Negative Examinations	Total No. of Consecutive Negative Examinations	No. of Consecutive Negative Examinations after Treatment
	Begun	Ended	Total Emetine			
III	Aug. 8	Aug. 20	12.75 grs.	Aug. 19 to Sept. 12 ... Relapse (Sept. 13)	17	16
	Sept. 14	Oct. 4	22.5 grs.	Sept. 18 to Oct. 10 ...	18	5
VIII	June 27	July 26	17.25 grs.	July 13 to Aug. 8 ... Relapse (Aug. 9)	15	5
	Aug. 8	Aug. 16	3.25 grs.	Aug. 14 to Aug. 22 ... Relapse (Aug. 23)	7	5
	Aug. 25	on 3rd course and still positive when transferred.				
XI	June 23	July 31	15.9 grs.	July 13 to Aug. 11 ... Relapse (Aug. 16)	16	6
	Aug. 8	Aug. 31	4.5 grs.	Aug. 23 to Sept. 19 ... Relapse (Sept. 20)	19	13
	Sept. 21	Oct. 16	37 grs.	Sept. 22 to Nov. 29 ... (continuing)	46	28
XX	July 11	Aug. 22	40.5 grs.	July 24 to Aug. 23 ... Relapse (Aug. 24)	23	1
	Aug. 28	Sept. 10	18 grs.	Aug. 30 to Sept. 20 ...	17	9
XXV	Aug. 9	Aug. 18	6.75 grs.	Aug. 15 to Sept. 7 ... Relapse (Sept. 12)	10	7
	Sept. 13	Sept. 26	18.5 grs.	Sept. 16 to Nov. 22 ...	28	22
XXXV	Aug. 18	Aug. 28	6 grs.	Aug. 22 to Sept. 5 ... Relapse (Sept. 6)	8	5
	Sept. 11	Oct. 10	52.5 grs.	Sept. 13 to Oct. 10 ...	20	0
LII	Oct. 4	Oct. 19	19.5 grs.	Oct. 7 to Oct. 17 ... Relapse (Oct. 20)	5	0
	Nov. 6	Nov. 20	19.5 grs.	Nov. 10 to Nov. 29 ... (continuing)	14	6
LV	Oct. 6	Oct. 30	24 grs.	Oct. 20 to Oct. 27 ... Relapse (Oct. 28)	7	0
	Nov. 23	On 2nd course alcrest a.				
LVI	Oct. 6	Oct. 23	19 grs.	Oct. 13 to Oct. 28 ... Relapse (Oct. 31)	7	4
	Nov. 6	Nov. 19	19.5 grs.	Nov. 7 to Nov. 27 ... (continuing)	17	6
LVIII	Oct. 15	Nov. 7	24 grs.	Oct. 27 to Nov. 6 ... Relapse (Nov. 7)	7	0
	Nov. 15	On methyl emetine.				
LIX	Oct. 27	Nov. 8	18 grs.	Oct. 30 to Nov. 15 ... Relapse (Nov. 17)	10	4
	Nov. 27	On 2nd course alcrest a.				

TABLE III—⁶ Relapsing Cases.—*Continued.*

Case No.	Treatment with Alcresta			Period of Consecutive Negative Examinations	Total No. of Consecutive Negative Examinations	No. of Consecutive Negative Examinations after Treatment
	Begun	Ended	Total Emetine			
LXIII	Oct. 20	Nov. 15	37.5 grs.	Nov. 7 to Nov. 10 ... Relapse (Nov. 13)	4	0
	Nov. 15	On methyl	emetine.			
LXIV	Oct. 19	Nov. 8	30 grs.	Oct. 24 to Nov. 6 ... Relapse (Nov. 8)	8	0
	Nov. 9	On 2nd course	alcrest a.			
LXIX	Nov. 4	Nov. 18	19.5 grs.	Nov. 6 to Nov. 20 ... Relapse (Nov. 21)	10	1
	Nov. 23	On 2nd course	alcrest a.			

CASE XXXV was discharged immediately after a second 'course' and might be considered in the first section with those cases discharged immediately after their first treatment.

CASE III relapsed after 17 negative examinations, 16 of them after treatment. He then had a further 'course' and 18 consecutive negative examinations were made on his faeces, but only 5 of these were subsequent to treatment, and in view of the long period that passed safely before his first relapse, this second negative period is not conclusive evidence of his having been cured. He was discharged.

CASE XX relapsed after 23 negative examinations, only one of them subsequent to treatment. He then had a further course, and 17 negative examinations were recorded. Nine of these were subsequent to treatment, and it is possible, though by *no means certain*, that he would not have relapsed again.

Cases XI and XXV are, on the other hand, as safely established 'cures' as we can hope to guarantee by observation over a necessarily limited period.

CASE XI relapsed after 16 negative examinations, 6 of them after treatment. He then had a second 'course' of alcresta, and 19 negative examinations were recorded. He relapsed a second time. A third course of alcresta seems finally to have cleared him up, however, since he has had 46 consecutive negatives, 28 of them since treatment stopped. He is still under examination.

CASE XXV relapsed after 10 negatives, 7 of them after treatment. A second course was followed by 28 negatives, 22 of them after treatment. He is still under examination.

Cases LII, LV, LVI, LIX, and LXIX have become 'negative' on a second 'course' of alcresta; LII and LVI, which have finished

their course, remain negative in the meantime, though it is too soon to be confident that they will not relapse.

Cases LVIII and LXIII were put on methyl emetine when it was found that a first course of alcresta had not cleared them up.

Though we are dealing here with much smaller numbers, a comparison of the incidence of relapses after a second treatment brings out the same point as does Table II, i.e. that the weeks immediately following treatment are critical.

TABLE IV.

	Negative	Positive
Of 9 cases that finished a 2nd treatment	8	1
Of 8 cases, hitherto negative, observed at the end of the 1st week after treatment	6	2
Of 2 cases, hitherto negative, observed at the end of the 2nd week after treatment	2	0
Of 2 cases, hitherto negative, observed at the end of the 3rd week after treatment	1*	1

* This negative case was observed to be negative still at the end of the 4th, 5th and 7th weeks.

Of two cases that finished a *third treatment*, both were negative at the end. By the end of the first week one had relapsed. This relapsing case belongs rightly, therefore, to the category of refractory cases, considered below. The negative case remained negative at the end of the second, third, fourth, fifth, sixth and seventh weeks.

(b) In this 'refractory' group there are four cases (IV, VI, XXIII, and XXXI) which have so far defied treatment with alcresta. One of them has been mentioned in Table IV as relapsing after a second and third treatment; the other three have not been included in Tables III and IV, as their treatment has been practically continuous. Cases VI, XXIII and XXXI have now been put on methyl emetine, kindly provided by Dr. H. H. Dale, F.R.S., of the Medical Research Committee; and it remains to be seen whether emetine in this form will effect a cure.

IV. SUMMARY AND CONCLUSIONS

Seventy-six cases harbouring the cysts of *Entamoeba histolytica* have been treated with emetine in the form of alcresta ipecac. given orally.

• Of these eighty-one—

- 13 left hospital immediately treatment stopped.
- 12 have not yet finished a first course of alcresta.
- 38 have not relapsed under observation subsequent to treatment.
- 14 have relapsed, *but at least four of these have subsequently cleared up on a second or third treatment*, and some of the remainder may yet do so.
- 4 are completely unaffected by the treatment.

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We have purposely cast Tables I and III in a form similar to that adopted by Dobell (1916) in a recent paper on the 'Incidence and Treatment of *Entamoeba histolytica* Infection at Walton Hospital,' in order that a comparison may more easily be made between the results of alcresta treatment and those of Dale's emetine bismuth iodide on the one hand* and of emetine hydrochloride on the other. (See also Jepps (1916)). While we cannot pretend to rival the amazing results obtained by the use of the biniodide at Walton Hospital (of twenty-five men treated with biniodide of emetine and bismuth *none* relapsed under observation)—our results do compare very favourably with those obtained by the hypodermic injection of emetine hydrochloride—70 per cent. of the cases at Walton Hospital relapsed after this treatment (Dobell), and 57 per cent. at the Kitchener Hospital, Brighton (Jepps).

Our acknowledgments are due to Messrs. Carter, Matthews and Malins Smith, who shared the routine work of the daily examinations with us in the laboratory of the Tropical School, and to Captain Llewellyn Morgan, R.A.M.C. (T.), and Dr. Abram, for their co-operation in the work at the Tropical School Auxiliary Military Hospital and Royal Infirmary respectively.

* It should be noted that of the cases recorded in the present paper only a small proportion, 14, have so far been on a 'standard' course of $1\frac{1}{2}$ grs. emetine daily for a fortnight. The cases at Walton were all on a standard course of 3 grains emetine bismuth iodide (containing 1 gr. emetine) daily for twelve days.

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THE PROTOZOAL FINDINGS IN NINE HUNDRED AND TEN CASES OF DYSENTERY EXAMINED AT THE LIVER- POOL SCHOOL OF TROPICAL MEDICINE FROM MAY TO SEPTEMBER, 1916

(FIRST REPORT)

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I. MATERIAL

From May 1st to September 30th, 1916, protozoological examinations were made at the Liverpool School of Tropical Medicine of the stools of 910 patients suffering from dysentery and related diseases. The total number of examinations was 4,334, and the results of these examinations are set forth in the present report.*

*Since 1 January, 1916, 2,162 cases of dysentery have been examined at the School and have received 8,158 examinations. Of these 1,305 cases, on which 3,824 examinations were made, have been reported on already by Fantham (1916). The 910 examined since 1 May include 53 of the previous report. Their inclusion is due to the fact that these patients resided in hospital from April to May, and also because in some instances they provided new infections after the date of the first report.

The material for examination was sent in from four different hospitals, viz., Tropical School Auxiliary Military, Mill Road Infirmary, 1st Western General (Fazakerley), and Highfield Military; one convalescent hospital, viz., Woolton; and two camps, viz., at Birkenhead and at Litherland. All these hospitals and camps are in the area of the Western Command. It may be stated at once that of these the Tropical School Auxiliary Military Hospital was the most important. On account of its proximity to the laboratory it was visited daily. Its cases were thus kept under closer supervision and subjected to more frequent examination than was possible elsewhere. Although it furnished only 37 per cent. of all the cases examined, yet on these cases 70 per cent. of all the examinations made were conducted. Full details on these points for all the hospitals are given later.

II. METHODS

Samples of stools which had been passed between 10 p.m. and 10 a.m. were collected, sent to the Tropical School Laboratory and examined as soon after arrival as possible. The examination was always completed on the day when the samples were sent. Two separate drops of normal saline were placed on a slide and a small quantity of the faeces to be examined rubbed into each of these to form an emulsion. Two preparations were thus obtained on one slide. They were examined under the 1/6 in. objective and No. 2 ocular. For cysts which could not be accurately determined by means of the above combination, the 1/12 in. oil immersion objective was used. In exceptionally difficult cases a further preparation was made in iodine (Lugol's solution), though this did not always render the determination easier. When very scanty infections or cases difficult to determine were encountered as many as five or six preparations or even ten or twelve were made, no limit, other than that enforced by time, being put upon the number of preparations examined. The time occupied in examination varied within wide limits; sometimes half an hour or longer was spent on a single stool, but the average time was of course less than this.

III. ANALYSIS OF RESULTS

The organisms producing the majority of the infections, and upon which our observations were made, are:—*Entamoeba histolytica*, Schaudinn; *Entamoeba coli* (Lösch); *Giardia* (*Lamblia*) *intestinalis* (Lambl); *Trichomonas intestinalis*, Leuckart, and *Chilomastix* (*Tetramitus*) *mesnili* (Wenyon).

The results obtained are as follows:—

Total number of cases examined ... 910

Number of cases having protozoal
infections ... 402 or 44·2 per cent.

In Table I an analysis of the 402 positive cases is given.

TABLE I

	Number of cases infected	Percentage of total cases examined	Pure infections	Mixed infections
<i>E. histolytica</i>	94	10·3	41	53
<i>E. coli</i>	231	25·4	142	89
<i>G. intestinalis</i>	169	18·6	106	63
<i>T. intestinalis</i>	11	1·2	2	9
<i>C. mesnili</i>	25	2·7	4	21
<i>Amoeba limax</i>	2	—	—	—

In numerous instances multiple infections of the Protozoa concerned occurred, and such double or triple infections found during the investigation are indicated in the following list:—

The double infections were:—

E. histolytica and *E. coli* in 27 cases.

E. histolytica „ *G. intestinalis* in 6 cases.

E. histolytica „ *T. intestinalis* „ 2 „

E. histolytica „ *C. mesnili* „ 4 „

E. coli and *G. intestinalis* in 36 cases.

E. coli „ *T. intestinalis* „ 2 „

E. coli „ *C. mesnili* „ 7 „

G. intestinalis and *T. intestinalis* in 1 case.

G. intestinalis „ *C. mesnili* „ 1 „

The triple infections were :—

<i>E. histolytica</i> , <i>E. coli</i> and <i>G. intestinalis</i>	in 8 cases.
<i>E. histolytica</i> , <i>E. coli</i> and <i>T. intestinalis</i>	„ 2 „
<i>E. histolytica</i> , <i>G. intestinalis</i> and <i>C. mesnili</i>	„ 4 „
<i>E. coli</i> , <i>G. intestinalis</i> and <i>C. mesnili</i>	„ 5 „
<i>E. coli</i> , <i>G. intestinalis</i> and <i>T. intestinalis</i>	„ 2 „

In the above lists the double infections and triple infections are quite separate, since in no case has a triple infection been included as a double infection also.

IV. COMPARISON WITH THE RESULTS OF OTHER WORKERS

Several other investigators have published results of examinations of dysenteric patients for intestinal protozoa. In Table II are given in parallel columns the results of (1) the present examinations which are all dysenteric cases; (2) Wenyon's (1915) examinations of 556 dysenteric cases; (3) Dobell's (1916) examinations of 200 patients from Egypt and Gallipoli, 90 of whom were dysenteric cases and 110 non-dysenteric cases; (4) Jepps' (1916) examinations of 426 intestinal cases invalided from the Mediterranean Expeditionary Force, of whom some only were diagnosed as dysenteric cases; and (5) Smith and Matthews' (1917) examinations of 250 non-dysenteric patients invalided from various regions. The figures given are percentages :—

TABLE II

	Present Report	Wenyon (1915)	Dobell (1916)	Jepps (1916)	Smith and Matthews (1917)
	1	2	3	4	5
<i>E. histolytica</i> ...	10.3*	10.8	11.0	7.7	8.0
<i>E. coli</i> ...	25.4	39.0	40.9	25.8	19.2
<i>G. intestinalis</i> ...	18.6	16.0	19.5	19.0	8.0
<i>T. intestinalis</i> ...	1.2	1.6	2.4	—	1.7
<i>C. mesnili</i> ...	2.7	0.5	7.8	—	2.0

* The percentage of *E. histolytica* infections recorded in the present report is much larger than that shown by Fantham (1916).

When we consider that the figures in columns 3, 4, and 5 are the results of observations made on patients of whom only a proportion were invalided home as dysenterics, it is perhaps surprising that they should so nearly approach the figures given in columns 1 and 2. Each investigation shows that the protozoa occur in the following order of frequency, beginning with the commonest:—(1) *E. coli*; (2) *G. intestinalis*; (3) *E. histolytica*; (4) *C. mesnili*; and (5) *T. intestinalis*. To this there are only two minor exceptions:—(1) Smith and Matthews show *E. histolytica* equally common with *G. intestinalis*, and (2) Wenyon finds *T. intestinalis* commoner than *C. mesnili*. Further the figures are in some cases surprisingly similar, e.g. *E. histolytica* only shows a range of 7·7 to 11, and in the first four columns *G. intestinalis* only ranges from 16·0 to 19·5. There are much greater differences between the percentages of *E. coli* found, the range being from 19·2 (or ignoring the fifth column, 25·4) to 40·9. The meaning of this is not clear, and at present it seems useless to speculate on it.

V. DISTRIBUTION OF CASES

In Table III is shown the distribution of the cases among the various hospitals and camps:—

TABLE III

Hospital or Camp	Number of cases examined	Total number of examinations made	Total infected cases	Cases infected with <i>E. histolytica</i>	Cases infected with <i>E. coli</i>	Cases infected with <i>G. intestinalis</i>	Cases infected with <i>T. intestinalis</i>	Cases infected with <i>C. mesnili</i>
Tropical School Auxiliary ...	341	3054	180	55	103	81	8	13
Mill Road Infirmary	261	701	106	15	62	45	1	5
First Western General ...	88	106	22	5	11	7	0	2
Highfield ...	52	98	17	4	8	7	0	2
Woolton ...	57	140	25	3	15	12	2	2
Birkenhead ...	54	116	21	4	17	9	0	1
Litherland ...	57	119	31	8	15	8	0	0
Total ...	910	4334	402	94	231	169	11	25
Percentage of all cases examined ...	—	—	44·2	10·3	25·4	18·6	1·2	2·7

It is obvious that the percentage (16.1) of *E. histolytica* cases is unusually high at the Tropical School Auxiliary Military Hospital. This we believe to be due to two causes. Firstly the cases sent to the Tropical School are to some extent selected cases. During the period of this report thirty-one cases were admitted from the 2nd Western General Hospital, all of which had a history of an infection with *E. histolytica*. Among these we found that eighteen were still positive for *E. histolytica*. If these cases be excluded the percentage of *E. histolytica* cases in the Tropical School Auxiliary Hospital falls from 16.1 to 11.9. Besides these a small number of known *E. histolytica* cases were admitted from other hospitals. Secondly the higher percentage is also due to the fact that the cases in the Tropical School Auxiliary Hospital were examined more often than those in the other hospitals. It is shown later in this report that patients negative on the first and second examinations may nevertheless have an infection of *E. histolytica*. Thus it is necessary, in order to find all the infections, that the stool of each patient should be examined several times. We give later reasons for thinking it necessary that at least three examinations should be made of each case. This standard was most nearly reached in the Tropical School Auxiliary Hospital, and therefore there were probably fewer infections of *E. histolytica* missed than in the cases from the other hospitals.

Special reference must here be made to the cases of *E. histolytica* found in the two camps as shown in Table III. The men in these camps were supposed to have been through all the necessary treatment to fit them for active service. Yet we found at Birkenhead 7.4 per cent. and at Litherland 14 per cent. of the cases examined were carriers of *E. histolytica*. Stress is not laid upon the actual percentage when such small numbers are concerned, but we think it important to record the fact that in each camp were found an appreciable number of men infected with *E. histolytica*. It seems probable that in the conditions of active service each of these men might become a source of danger to other men. There is also the likelihood of the disease developing to a more serious stage in the 'carrier' himself. We have no experience to guide us to a judgment as to how far either of these possibilities is a serious danger in a temperate climate, but the possibilities exist and the danger may be grave.

VI. DETAILS OF EXAMINATIONS MADE

The actual numbers of examinations made were as follows:—

- 910 cases had at least one examination each.
- 767 cases had at least two examinations each.
- 519 cases had at least three examinations each.
- 293 cases had at least four examinations each.
- 202 cases had at least five examinations each.
- 156 cases had at least six examinations each.

Each fresh examination resulted in an addition to the total number of cases of Protozoal infection found. A case which showed single infection at the first examination frequently proved on second and third examinations to have double or multiple infection; also a case which showed initial double infection sometimes proved later to have a multiple infection. For instance, *E. coli* was often found in cases already known to be infected with *G. intestinalis*, and in other cases where *E. histolytica* and *G. intestinalis* were already known to be present. Table IV shows exactly the increase with repeated examinations of (a) the total number of infected cases, (b) cases infected with *E. histolytica*; (c) with *E. coli*; and (d) with *G. intestinalis*.

TABLE IV

Number of cases examined	Examination	Total infected cases	Percentage of total cases examined	Cases infected with <i>E. histolytica</i>	Percentage of total cases examined	Cases infected with <i>E. coli</i>	Percentage of total cases examined	Cases infected with <i>G. intestinalis</i>	Percentage of total cases examined
		(a)		(b)		(c)		(d)	
910	First	259	28.5	57	6.3	121	13.3	108	11.9
767	Second	357	39.2	77	8.4	185	20.3	144	15.8
519	Third	387	42.5	88	9.6	208	22.8	155	17.0
293	Fourth	396	43.5	91	10.0	218	24.0	159	17.4
202	Fifth	399	43.8	92	10.1	221	24.2	161	17.7
156	Sixth	401	44.1	94	10.3	223	24.5	164	18.0
Ultimate results (vide Table I)		402	44.2	94	10.3	231	25.4	169	18.6

The additional case occurring in the totals of column (a) was not recorded as positive until the eighth examination, when an

infection of *E. coli* was discovered. In the same column it is shown that nine new infections were found on the fourth examination. These consisted of two infections of *E. histolytica*, five of *E. coli*, and two of *G. intestinalis*. The three new infections shown on the fifth examination were one of *E. coli* and two of *G. intestinalis*. The two new infections on the sixth examination were one of *E. histolytica* and one of *E. coli*.

On account of its known pathogenicity the infections with *E. histolytica* are the most important in practice, and special consideration must be given to the figures in column (b), Table IV. We may obtain guidance from them as to the number of examinations which ought to be made on all patients in order to make sure that all cases of *E. histolytica* are found. It is at once obvious that *three* examinations is an irreducible minimum. *At least* three are absolutely necessary. If only two examinations had been made on these men, seventeen cases of *E. histolytica* would have been missed. This forms 1·9 per cent. of all the cases examined and 18 per cent. of all the *E. histolytica* cases found. If only three examinations had been made, six cases of *E. histolytica* would have escaped detection. This number is 0·7 per cent. of all the cases examined and 6·4 per cent. of all the *E. histolytica* cases found. To miss such a percentage becomes a serious matter when large numbers are being examined, and to diagnose from *fewer* examinations than three is clearly unsatisfactory.

In column (c), Table IV, will be found the figures for the number of cases infected with *E. coli*. It will be seen from them that if only three examinations had been made, twenty-three infections of *E. coli* would have escaped detection, i.e. 2·5 per cent. of the total number of cases examined, or 10 per cent. of all the *E. coli* cases found. Further, the table shows that eight of these twenty-three were discovered after the sixth examination. These were found in the following order:—Two on the seventh examination, three on the eighth, one on the twentieth, one on the twenty-fourth, and one on the thirty-fifth.

In column (d), Table IV, the figures are given for the number of cases infected with *G. intestinalis*. Had only three examinations been made fourteen infections would have been missed, i.e. 1·5 per cent. of the total cases examined or 8·3 per cent. of all the cases having

an infection with *G. intestinalis*. Five of these were detected after the sixth examination, one on the tenth examination, one on the twelfth, one on the thirteenth, one on the nineteenth, and one on the twenty-eighth.*

Although we have given in our table figures relating to the commoner protozoa only, it may be mentioned that infections of *C. mesnili* and *T. intestinalis* also were found after the sixth examination. In four cases, each infected with *E. histolytica*, *C. mesnili* was first observed on the sixteenth, seventeenth, twenty-third and forty-fourth examination, respectively. In one case *T. intestinalis* was not detected until the eleventh examination.

It is obvious that these findings could only have been obtained by continued observation of the cases concerned. The importance of daily examinations was recognised at the outset, and it was our original aim to examine patients every day over as long periods as possible, but it was found impracticable to follow this procedure with all infected cases. It has been followed principally with the *E. histolytica* cases, and particularly with those found in the Tropical School Auxiliary Hospital. We have, therefore, a relatively small number of cases on whom prolonged observations have been conducted. Among these were some who had double or multiple infections, and thus the long record of a case having, for example, an infection of *E. histolytica* and *E. coli* proved doubly valuable and interesting. We have, however, carried out daily examinations on a few selected cases having an infection of *E. coli* or *G. intestinalis* only.

The records obtained suggest certain points of interest which we will mention here only briefly, as it is anticipated that a fuller discussion will be given in a subsequent paper when we have a greater number of facts at our disposal.

The appearance of the cysts of *E. coli* on a seventh or eighth examination is not surprising, since the records of a number of cases show that a period of at least fourteen days may occur when no evidence of the protozoon can be found in the stools.† These

*With regard to this case it should be pointed out that the infection never recurred, and the single appearance of the parasite suggests the possibility of some confusion having arisen before the samples reached the laboratory.

†On account of the difficulty of getting specimens daily without intermission, the 'negative periods' of a fortnight usually included 10 to 12 examinations instead of 14.

records are those of patients who were also infected with *E. histolytica* and who were receiving emetine treatment in the form of 'alcresta ipecac.' It is possible that the drug had an inhibitory effect on *E. coli*, and this might partly account for the length of the 'negative period' indicated. Some of the records certainly point in this direction, but in others *E. coli* persisted irrespective of the treatment given for *E. histolytica*. In the records of pure *E. coli* infections, where, of course, no alcresta was administered, negative intervals of seven days are not uncommon. In a few cases 'negative periods' of ten to seventeen days have occurred, and two cases have been observed whose records are sufficiently long to suggest that the infection had disappeared entirely.

The remarks, which have been made regarding a few special features shown by the records of *E. coli* cases, apply to a certain extent to cases infected with *G. intestinalis*. As a rule, however, negative intervals were less common, and generally shorter in cases infected with *G. intestinalis* than in *E. coli* cases. Certain ratios bearing upon this point will be given later. In cases having an infection of *G. intestinalis* we have obtained periods of ten days during which neither the free forms nor the cysts of this parasite were found in the stools. Very rarely did a longer 'negative period' occur, but in one case, where a scanty infection had been observed the record shows a period of thirty-three days during which twenty-six examinations were made before the cysts were again found. Generally, we have found infections of *G. intestinalis* to be fairly heavy and remarkably persistent. A number of the cases infected with *G. intestinalis* were also infected with *E. histolytica*, and the records of these cases show that the former protozoon remained unaffected by alcresta.

A few records of cases infected with *C. mesnili* are sufficiently long to deserve notice. These indicate that periods of from seven to sixteen days may occur during which neither the flagellates nor the cysts can be found in the stools of the infected patients. That this protozoon should not be found till the forty-fourth examination in one particular case may seem a little surprising, but it must be pointed out that during the period of the forty-four examinations there were sixteen days at irregular intervals when no examinations were made, and it is possible that the protozoon escaped notice on

one or more of these occasions. Infections of *C. mesnili* were generally fairly persistent, although in one case there seemed to be a complete disappearance of the parasite.

The infections we found of *T. intestinalis* were as a rule rather scanty, and in most instances of comparatively short duration. One case, however, is worthy of special notice. The infection persisted over a considerable period, and the daily record showed four distinct intervals—two of thirteen days, one of fourteen days, and one of nine days—during all of which no flagellates were detected in the stools.

We give in Table V the number of examinations conducted on the cases infected with *E. histolytica*, *E. coli* and *G. intestinalis*. The table also indicates the number of times the organisms were found.

TABLE V

Hospital or Camp	<i>E. histolytica</i> cases		<i>E. coli</i> cases		<i>G. intestinalis</i> cases	
	Number of Examinations made	Number of times found	Number of Examinations made	Number of times found	Number of examinations made	Number of times found
Tropical School Auxiliary	1446	304*	1448	487*	934	467*
Mill Road Infirmary ...	49	20	197	93	151	97
First Western General ...	6	5	17	11	9	8
Highfield	9	4	16	10	15	10
Woolton	12	7	42	23	44	31
Birkenhead	12	12	42	23	20	14
Litherland	18	11	36	20	15	11
Total	1552	363	1798	667	1188	638
Ratio	4.3 : 1		2.7 : 1		1.9 : 1	

* Of the 304 times that *E. histolytica* was found in cases from the Tropical School Auxiliary Hospital, the cysts occurred 299 times and the amoebae 11 times. In the case of infections with *E. coli*, the cysts were detected 476 times and the amoebae 18 times. In *G. intestinalis* infections, the cysts were discovered 456 times and the flagellates 20 times.

That the *E. coli* cases should have received so many examinations is due to the frequent occurrence of this entamoeba with *E. histolytica* or with *G. intestinalis*, or with both, and also to the fact that it is in itself the commonest of all the protozoa with which we were dealing.

It will be observed that the cases infected with *E. histolytica* were examined 1,552 times in all, and the parasites found on 363 occasions, or, on an average, once in every five examinations made. The correct ratio is 1 : 4·3. The infrequency of positive examinations is of course due to the fact that immediately the parasite was detected, the infected case was placed under treatment which generally resulted in a number of subsequent negative examinations. If the figure for the Tropical School Auxiliary Hospital be omitted, the ratio obviously becomes higher owing to the fact that it was impossible to follow the cases in the other hospitals so carefully, and, therefore, fewer examinations of these cases were obtained. The *E. coli* cases were examined 1,798 times and the organism found on 667 occasions. The ratio is here 1 : 2·7, which means approximately that the protozoon was detected once in every three examinations performed. The cases infected with *G. intestinalis* received 1,188 examinations and the parasite was found 638 times, i.e. once in every two examinations, or correctly 1 : 1·9. These ratios receive striking confirmation from those obtained by Smith and Matthews (1917). Their figures are for all *E. histolytica* cases found 1 : 3·1, for untreated *E. histolytica* cases 1 : 2·35, for *E. coli* 1 : 2·7, and for *G. intestinalis* 1 : 1·75. It is clear from Tables IV and V that our ratios are *no more than an approximate guide* to the number of examinations necessary for the detection of infections, but those referring to *E. coli* and *G. intestinalis* are of considerable interest in indicating the relative frequency of the occurrence of these organisms in the stools of infected patients.

VII. GRAPHICAL PRESENTATIONS OF RESULTS

In fig. 1, we give in the form of curves the results of our continued examination of the cases dealt with in this report. The figures on which the curves are based have already been presented in Table IV, and the curves serve to make clearer the gradual

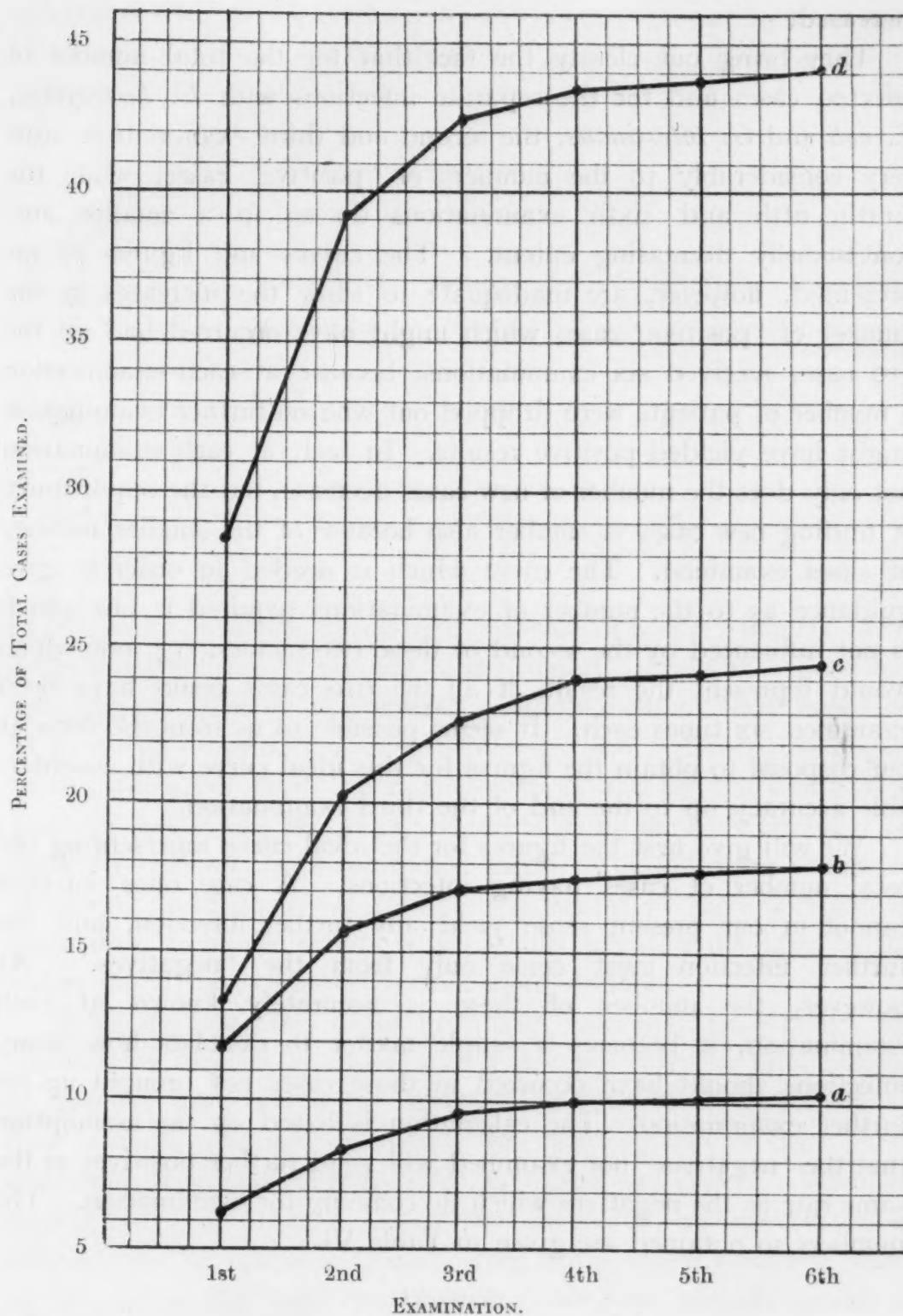


FIG. 1. Curves showing the percentage increase in infections at each of six examinations for (a) cases infected with *E. histolytica*; (b) cases infected with *G. intestinalis*; (c) cases infected with *E. coli*, and (d) total infected cases.

increase of infections of all sorts as the number of examinations is increased.

They bring out clearly the fact that for the total number of infected cases and for the separate infections with *E. histolytica*, *E. coli* and *G. intestinalis*, the second and third examinations add very considerably to the numbers of 'positive' cases, while the fourth, fifth and sixth examinations do so to a smaller and continuously decreasing extent. The curves and figures so far presented, however, are inadequate to show the increases in the number of 'positive' cases which might have occurred had all the 910 cases received six examinations, because at each examination a number of patients were dropped out who on further examination might have yielded positive results. In fact, at each examination not only does the number of new cases decrease, but the opportunity of finding new cases is smaller also because of the smaller number of cases examined. The curve which is needed in order to give guidance as to the number of examinations required is one which is not influenced by the second of these two factors, e.g. one which would represent the results if all the 910 cases could have been examined six times each. It seems possible to us from the data at our disposal to obtain the figures for this ideal curve with considerable accuracy up to the end of the third examination.

We will give first the figures for the ideal curve representing the total number of cases having infections. A case once infected cannot in our present sense yield any further infection, and the further infection must come only from the 'negatives.' As however, the number of these is accurately known at each examination, it becomes a simple matter to calculate how many infections should have occurred in those cases not brought up for further examination. The calculation is based on the assumption that the 'negatives' not examined will yield further positives at the same rate as the negatives which do come up for examination. The numbers so obtained are given in Table VI.

TABLE VI

Examination			Total infected cases calculated on basis of 910 examined each time	Percentage of total cases examined	Additional infections at each examination (calculated)	Additional infections actually found from diminishing numbers examined
First	259	28.5	259	259
Second	374	41.1	115	98
Third	429	47.1	55	30

We will now consider the figures for the ideal curve for infections with *E. histolytica*, i.e. the curve representing the results if all 910 cases had been examined three times. The figures are given in Table VII.

TABLE VII

Examination	Cases infected with <i>E. histolytica</i>	Percentage of total cases examined	Additional infections at each examination (calculated)	Additional infections actually found from diminishing numbers examined
First	57	6.3	57	57
Second	80	8.8	23	20
Third	99	10.9	19	11

We have been able to follow the history of a small number of the cases since the date of the end of this report. Some have gone to a convalescent home in this district, and their stools have been sent in to us for examination since September 30th. Others have gone to the dysentery depôt at Barton-on-Sea, and by the kindness of Captain Banks and Mr. W. O. Redman King we have obtained the reports of examinations conducted there. The following results are of interest, and go to substantiate the figures we have obtained by calculation as to the results which would accrue if the cases in this report could have received further examinations.

If all the 910 cases could have been examined three times, our calculations lead us to expect (see Table VI) that the total number of infected cases would be increased by forty-two. Of these we have actually obtained six among the small number of cases which received a third examination after the report was closed. When we confine ourselves to the *E. histolytica* cases only, we expect from our calculations (see Table VII) that eleven further cases would be found by the end of the third examination. We have already found, or have had reported to us, six *E. histolytica* cases among those who have received a third examination since the report was closed.

VIII. ORGANISMS OTHER THAN PROTOZOA

Finally, we may mention that infections other than protozoal were occasionally encountered. Six cases showed worms' eggs in the faeces; four patients were thus infected with *Trichiuris trichiura* (L.) (= *Trichocephalus dispar*, Rudolphi); one, with *Ascaris lumbricoides*, L., and one with *Ancylostoma duodenale*, Dubini. The organism described by Wenyon (1915) under the name 'I body' was found in five cases.

Our acknowledgments are due to Professor J. W. W. Stephens, Medical Officer in charge of the Tropical School Auxiliary Military Hospital, for his general interest in the work and for various helpful suggestions; to Professor Warrington Yorke, who has frequently given us the benefit of his experience in diagnosing difficult cases; and to Captain Llewellyn Morgan, R.A.M.C. (T.), who has facilitated our work at the Tropical School Hospital. We are also indebted to the sisters of this hospital for their willing co-operation in the daily collection of specimens. During the first few weeks of the period covered by this report we had the assistance, in carrying out the examinations, of Miss M. Pallis and Mr. W. Riddell, M.A., to whom we tender our thanks for their help.

NOTE.—While this paper was being prepared for the press, we had the privilege of seeing an advance copy of Mr. Clifford Dobell's report to the Medical Research Committee on examinations of dysenteric cases. On one important point treated in both his and our reports, we wish to make an observation. Mr. Dobell has laid stress upon the inadequacy of three examinations to detect a safe proportion of the cases of *E. histolytica*. We should like to state that in advocating three examinations as an *absolute minimum* we do not necessarily differ with Mr. Dobell who advocates six. We were aware of the desirability of more than three examinations, but thought it wise, since we know from practical experience that quite a considerable proportion of the patients obtain only one or two examinations, to advocate as a necessary first step toward improvement that each patient without exception should receive at least three examinations.

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NOTE ON THE 'ARNETH COUNT' IN HEALTHY ABORIGINAL CHILDREN OF NORTHERN AUSTRALIA

BY

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AND

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FROM THE AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE

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Previous work by us (1914) on Arneth counts of healthy white school children born and bred in North Queensland led to the interesting observation that the Arneth index showed a distinct increase when compared with that of normal individuals in Europe, being 74·5 as compared to 40·0.

In continuation of this investigation, opportunity was taken (1915) to estimate the Arneth index of natives—adults and children—of New Guinea.

The blood slides for this investigation were collected during a journey through the coastal districts of New Guinea, where yaws and malarial fever were endemic and more or less widely spread amongst the children examined.

The results showed that the Arneth index for adult natives corresponded closely to that of North Queensland school children of European descent, being 74·0, whereas that of the native children was considerably higher, namely 83·9.

A consideration of these figures led to the surmise that the greater shift in native New Guinea children resulted, in all probability, from the effects of active or latent malarial fever and yaws. Unfortunately none of the districts traversed were free from either of the two diseases, and it was therefore impossible to prove this surmise.

A visit to the Northern Territory of Australia afforded the desired opportunity. On Melville and Bathurst Islands, situated

off the North Coast of Australia, forty children were seen, all of whom proved healthy on examination; their spleens were not enlarged, and in none of the blood slides taken could malarial parasites be discovered, nor was there any evidence of yaws amongst them.

Arneth counts were therefore performed on these slides. The same technique was employed as in our previous work. Two sets of a hundred consecutive leucocytes were enumerated, and only counts considered where the two sets of figures agreed closely.

Table I contains the averages obtained for Arneth and differential counts of these children, and, for comparison, the corresponding figures for North Queensland school children and for natives of New Guinea, adults and children. There is a close agreement between the figures of the first three groups, whereas those for the native New Guinea children show the deviation from the European standard still more marked. This close agreement strengthens our conception that the alteration of the blood picture in North Queensland school children can be regarded as an outcome of climatic influences only.

Scott Macfie (1915) believes that 'it is probable that the abortive inoculations with malaria parasites . . . are sufficient to account for this shift in apparently healthy Europeans, without postulating the specific action of the climate on the white races living in the tropics.' He suggests, further, that 'the changes observed in the Arneth counts are due to toxæmia causing a destruction of the circulating polymorphonuclear leucocytes and a flooding of the blood with young cells liberated by the activity of the leucopoietic system.'

It is feasible that the changed Arneth picture in malarial fever can be accounted for by the reaction of the organism, and especially the blood-forming organs, to the parasitic invasion.

In the blood of the North Queensland school children and the native children of Bathurst and Melville Islands there is a distinct increase in the Arneth index. In both localities malarial fever and other parasitic infections can be excluded, and some cause, other than disease, must be sought for. The further increase in the case of the native New Guinea children, living in endemic areas, may be accounted for by infection.

We believe that the results of the Arneth counts of Northern

TABLE I

	Number	Arneth Classification per cent.					Arneth Index	Differential Counts per cent.					
		I	II	III	IV	V		Poly- morpho- nuclear neutro- phile	Transi- tionals	Large Mono- nuclear	Lympho- cytes	Eosino- philes	Mast cells
Northern Territory Children	39	25.8	45.8	23.3	4.8	0.3	71.6	52.3	2.0	1.2	33.9	10.5	0.1
North Queensland School Children (white) ...	150	32.5	42.0	20.6	4.5	0.4	74.5	56.1	4.2	2.4	29.5	7.7	0.04
New Guinea Adult Natives...	104	30.04	44.0	21.23	4.3	0.43	74.04	51.08	2.9	1.95	32.1	11.97	—
New Guinea Native Children	50	42.96	40.9	13.8	2.13	0.21	83.86	40.96	2.47	1.36	42.86	13.24	—
Normal Europeans ...	—	5.0	35.0	41.0	17.0	2.0	40.0*	65.70	6.8		20.25	2.4	0.5†

† Ehrlich Lazarus

* Arneth

Australian native children, living in a healthy district, form a link in the proof that climatic conditions in the tropics as such can be held responsible for the altered blood conditions of inhabitants of the tropics, and that it is not necessary to resort to endemic disease as an explanatory factor.

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Note on the 'Arneth Count' in Healthy Aboriginal Children of Northern Australia. By A. BREINL, and H. PRIESTLEY.

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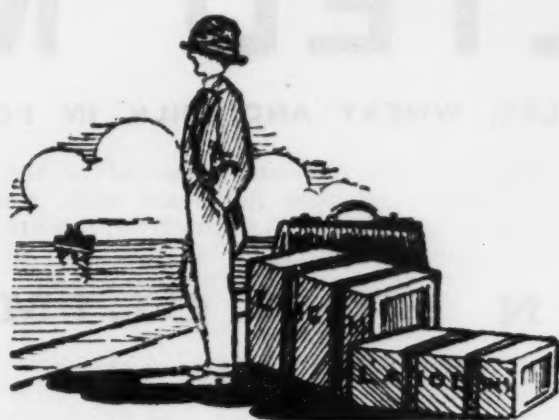
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B 10 Exchange Buildings, Liverpool

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Tropical Medicine*

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F.E.S., *Dutton Memorial Professor of Entomology*

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1902. (Indian Medical Service, retired). *Professor
of Tropical Sanitation*

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Assistant to the Cantab., D.P.H.
Physician - WILLIAM THOMAS PROUT, M.B., C.M.G.

4. At the Yellow Fever Research Laboratory of the School, Manaos

Director - - HAROLD WOLFERSTAN THOMAS, M.D.,
C.M.

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NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1917:—

Full Course begins 6 January. Advanced Course begins 1 June.

Diploma Examination, 2 April. Certificate Examination, 30 June.

Full Course begins 15 September.

Diploma Examination, 17 December.

These dates are subject to revision.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

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Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool:—

Diploma in Tropical Medicine

*Date of
Diploma*

1904 Augustine, Henry Joshua
1904 Bennett, Arthur King
1904 Bruce, William James
1904 Byrne, John Scott
1904 Clayton, Thomas Morrison
1904 Dalziel, John McEwen
1904 Dee, Peter
1904 Greenidge, Oliver Campbell
1904 Hehir, Patrick
1904 Khan, Saiduzzafar
1904 Laurie, Robert
1904 Maclurkin, Alfred Robert
1904 McConnell, Robert Ernest
1904 Nicholson, James Edward
1904 Philipson, Nicholas
1904 Sharman, Eric Harding
1904 Thomson, Frank Wyville
1904 Walker, George Francis Clegg

1905 Anderson, Catherine Elmslie
1905 Brown, Alexander
1905 Caldwell, Thomas Cathcart
1905 Critien, Attilio
1905 Hooton, Alfred
1905 Hudson, Charles Tilson
1905 Illington, Edmund Moritz

*Date of
Diploma*

1905 Macfarlane, Robert Maxwell
1905 Maddock, Edward Cecil Gordon
1905 Moore, James Jackson
1905 Nightingale, Samuel Shore
1905 Radcliffe, Percy Alexander Hurst
1905 Young, John Cameron

1906 Adie, Joseph Rosamond
1906 Arnold, Frank Arthur
1906 Bate, John Brabant
1906 Bennetts, Harold Graves
1906 Carter, Robert Markham
1906 Chisholm, James Alexander
1906 Clements, Robert William
1906 Dundas, James
1906 Faichnie, Norman
1906 Jeffreys, Herbert Castelman
1906 Mackenzie, Donald Francis
1906 Pailthorpe, Mary Elizabeth
1906 Palmer, Harold Thornbury
1906 Pearse, Albert
1906 Sampey, Alexander William
1906 Smithson, Arthur Ernest
1906 Taylor, Joseph van Someron
1906 Taylor, William Irwin
1906 Tynan, Edward Joseph

*Date of
Diploma*

1906 Watson, Cecil Francis
 1906 Willcocks, Roger Durant
 1906 Williamson, George Alexander

 1907 Allan, Alexander Smith
 1907 Allwood, James Aldred
 1907 Bond, Ashton
 1907 Branch, Stanley
 1907 Collinson, Walter Julius
 1907 Davey, John Bernard
 1907 Donaldson, Anson Scott
 1907 Fell, Matthew Henry Gregson
 1907 Gann, Thomas William Francis
 1907 Graham, James Drummond
 1907 Hiscock, Robert Carroll
 1907 Keane, Joseph Gerald
 1907 Kennan, Richard Henry
 1907 Kenrick, William Hamilton
 1907 Le Fanu, George Ernest Hugh
 1907 Mackey, Charles
 1907 Maddox, Ralph Henry
 1907 McCarthy, John McDonald
 1907 Raikes, Cuthbert Taunton
 1907 Ryan, Joseph Charles
 1907 Vallance, Hugh

 1908 Caverhill, Austin Mack
 1908 Crawford, Gilbert Stewart
 1908 Dalal, Kaikhusroo Rustomji
 1908 Dansey-Browning, George
 1908 Davidson, James
 1908 Dickson, John Rhodes
 1908 Dowdall, Arthur Melville
 1908 Glover, Henry Joseph
 1908 Greaves, Francis Wood
 1908 Goodbody, Cecil Maurice
 1908 Harrison, James Herbert Hugh
 1908 Joshi, Lemuel Lucas
 1908 Le Fanu, Cecil Vivian
 1908 Luethgen, Carl Wilhelm Ludwig
 1908 Mama, Jamshed Byramji
 1908 McCay, Frederick William
 1908 McLellan, Samuel Wilson
 1908 Pearce, Charles Ross
 1908 Schoorel, Alexander Frederik
 1908 Smith, John Macgregor
 1908 Stewart, George Edward
 1908 Tate, Gerald William
 1908 Whyte, Robert

 1909 Abercrombie, Rudolph George
 1909 Allin, John Richard Percy
 1909 Armstrong, Edward Randolph
 1909 Barrow, Harold Percy Waller
 1909 Beatty, Guy
 1909 Carr-White, Percy
 1909 Chevallier, Claude Lionel
 1909 Clark, William Scott
 1909 Cope, Ricardo
 1909 Fleming, William
 1909 Hanschell, Hother McCormick
 1909 Hayward, William Davey
 1909 Henry, Sydney Alexander
 1909 Innes, Francis Alexander

*Date of
Diploma*

1909 Jackson, Arthur Frame
 1909 Kaka, Sorabji Manekji
 1909 McCabe-Dallas, Alfred Alexander Donald
 1909 Meldrum, William Percy
 1909 Murphy, John Cullinan
 1909 Samuel, Mysore Gnananandaraju
 1909 Shroff, Kawasjee Byramjee
 1909 Thornely, Michael Harris
 1909 Turkhud, Violet Ackroyd
 1909 Webb, William Spinks
 1909 Yen, Fu-Chun

 1910 Brabazon, Edward
 1910 Castellino, Louis
 1910 Caulerick, James Akilade
 1910 Dowden, Richard
 1910 Haigh, William Edwin
 1910 Hamilton, Henry Fleming
 1910 Hefferman, William St. Michael
 1910 Hipwell, Abraham
 1910 Homer, Jonathan
 1910 Houston, William Mitchell
 1910 James, William Robert Wallace
 1910 Johnstone, David Patrick
 1910 Korke, Vishnu Tatyaji
 1910 Macdonald, Angus Graham
 1910 Macfie, John Wm. Scott
 1910 Manuk, Mack Walter
 1910 Murison, Cecil Charles
 1910 Nanavati, Kishavlal Balabhai
 1910 Naus, Ralph Welty
 1910 Oakley, Philip Douglas
 1910 Pratt, Ishmael Charles
 1910 Sabastian, Thiruchelvam
 1910 Shaw, Hugh Thomas
 1910 Sieger, Edward Louis
 1910 Sousa, Pascal John de
 1910 Souza, Antonio Bernardo de
 1910 Waterhouse, John Howard
 1910 White, Maurice Forbes

 1911 Blacklock, Breadalbane
 1911 Brown, Frederick Forrest
 1911 Chand, Diwan Jai
 1911 Holmes, John Morgan
 1911 Ievers, Charles Langley
 1911 Iles, Charles Cochran
 1911 Ingram, Alexander
 1911 Kirkwood, Thomas
 1911 Knowles, Benjamin
 1911 Liddle, George Marcus Berkeley
 1911 Lomas, Emanuel Kenworthy
 1911 Mackarell, William Wright
 1911 MacKnight, Dundas Simpson
 1911 Mascarenhas, Joseph Victor
 1911 Murray, Ronald Roderick
 1911 Oluwole, Akidiya Ladapo
 1911 Rao, Koka Ahobala
 1911 Sinton, John Alexander
 1911 Tarapurvala, Byramji Shavakshah
 1911 Taylor, John Archibald
 1911 Woods, William Medlicott

*Date of
Diploma*

1912 Aeria, Joseph Reginald
1912 Anderson, Edmund Litchfield
1912 Borle, James
1912 Bowie, John Tait
1912 Brassey, Laurence Percival
1912 Christie, David
1912 Dillon, Henry de Courcy
1912 Dunn, Lillie Eleanor
1912 Hardwicke, Charles
1912 Jagose, Jamshed Rustomji
1912 Kochhar, Mela Ram
1912 McGusty, Victor William Tighe
1912 Milne, Arthur James
1912 Mitra, Manmatha Nath
1912 Myles, Charles Duncan
1912 Pelly, Huntly Nevins
1912 Prasad, Bindeshwari
1912 Prentice, George
1912 Ross, Frank
1912 Russell, Alexander James Hutchison
1912 Ruthven, Morton Wood
1912 Sandilands, John
1912 Seddon, Harold
1912 Smalley, James
1912 Strickland, Percy Charles Hutchison
1912 Watson, William Russel

1913 Austin, Charles Miller
1913 Banker, Shiavux Sorabji
1913 Becker, Johann Gerhardus
1913 Carrasco, Milton
1913 Clark, James McKillican
1913 Forsyth, Charles
1913 Grahame, Malcolm Claude Russell
1913 Grieve, Kelburne King
1913 Hargreaves, Alfred Ridley
1913 Hepper, Evelyn Charles
1913 Hiranand, Pandit
1913 Jackson, Oswald Egbert
1913 Khaw, Ignatius Oo Kek
1913 MacKelvie, Maxwell
1913 MacKinnon, John MacPhail
1913 Macmillan, Robert James Alan
1913 Mouat-Biggs, Charles Edward Forbes
1913 Noronha, John Carmel
1913 O'Connor, Edward
1913 Olubomi-Beckley, Emanuel

*Date of
Diploma*

1913 Pestonji, Ardeshir Behramshah
1913 Puttanna, Dodballapur Sivappa
1913 Reford, John Hope
1913 Smith, Edward Arthur
1913 Stewart, Samuel Dudley
1913 Walker, Frederick Dearden
1913 Wilbe, Ernest Edward
1913 Wilson, Hubert Francis
1913 Yin, Ulg Ba
1913 Young, William Alexander

1914 Arculli, Hassan el
1914 Chohan, Noormahomed Kasembha
1914 Connell, Harry Bertram
1914 Gerrard, Herbert Shaw
1914 Gimi, Hirji Dorabji
1914 Gwynne, Joseph Robert
1914 Hodgkinson, Samuel Paterson
1914 Jackson, Arthur Ivan
1914 Kaushash, Ram Chander
1914 Kelsall, Charles
1914 Luanco y Cuenca, Maximino
1914 Misbah, Abdul-Ghani Naguib
1914 Naidu, Bangalore Pasupulati
Balakrishna
1914 Rowe, John Joseph Stephen
1914 Roy, Raghu Nath
1914 Shiveshwarkar, Ramchandra Vishnu
1914 Sur, Sachindra Nath
1914 Talati, Dadabhai Cursedji
1914 Wilkinson, Arthur Geden
1914 Wright, Ernest Jenner

1915 Lobo, John Francis
1915 Madhok, Gopal Dass
1915 Pearson, George Howorth
1915 Swami, Karumuri Virabhadra
1915 Wood, John

1916 Barseghian, Mesroob
1916 Chaliha, Lakshmi Prasad
1916 Lim, Albert Liat Juay
1916 Lim, Harold Liat Hin
1916 Metzger, George Nathaniel
1916 Söderström, Erik Daniel
1916 Wheeler, Louis

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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ROBINSON, S. (1914). ‘The spleen in malaria.’ *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). ‘Enlargement of the spleen in malaria.’ *Journal of Pathometry*, Vol. I, pp. 1-20.

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